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**The pathophysiology of fluid removal during
haemodialysis : Implications for blood volume
monitoring and determination of dry weight**

Sandip Mitra

**A thesis submitted in fulfilment of the requirements of the
University of London for the degree of Doctor of Medicine**

**Supervised by : Dr Ken Farrington & Dr Andrew Davenport
Royal Free and University College Medical School**

**The programme of research was carried out in the Renal Unit,
Lister Hospital, Stevenage, Hertfordshire, UK**

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Structure of Thesis

Introduction and review of literature

This section introduces end stage renal failure, its prevalence, demographics and treatment in the renal population. A brief history of haemodialysis treatment and some key clinical issues contributing to dialysis mortality and morbidity today are discussed with particular emphasis on volume dependent hypertension and other cardiovascular aspects. Evolution of concepts in dry weight on dialysis, fluid pathophysiology in humans and its regulation during dialysis and blood volume based studies are reviewed to provide the essential knowledge base for understanding body responses to ultrafiltration during dialysis. Stating the aims and objectives of the current research concludes the section.

Methods

Details of methodology, machines and tools used for experimental studies have been described in this section. A short description of algorithms and systems required for simultaneous data acquisition are included as an important part of current research. Study designs and their rationale briefly alluded to here have been more extensively discussed in subsequent chapters.

Experimental Clinical Studies

This section has been arranged in separate chapters (describing individual experiments), which address specific aspects of monitoring fluid management. There are three main subdivisions. Chapters 1&2 investigate blood pressure measurements, bioimpedance determined fluid compartments and nutritional markers in relation to fluid management Chapters 3,4,5 & 6 describe relative blood volume responses to ultrafiltration and Chapters 7,8,9 & 10 describe experiments to directly measure absolute blood volume changes during dialysis, its relationship with other fluid compartments and relative blood volume changes. Finally it provides an insight into interesting pathophysiological responses to ultrafiltration during haemodialysis.

Overview

This section summarises the contribution of knowledge resulting from the current research and recommendations for further work.

Appendices

The appendices include additional material and demonstrate a number of derivations flowsheets used and practical examples of data analysis.

References

The pathophysiology of fluid removal during haemodialysis : Implications for blood volume monitoring and determination of dry weight

Abstract

The aim of the research was to characterise the pathophysiology of ultrafiltration (UF) during haemodialysis (HD) using a range of assessment tools. The hypothesis was that objective monitoring would facilitate accurate estimation of dry weight (DW) and help optimise UF.

Measurement timing, white-coat effect, UF prescription and volume status influenced blood pressure (BP) readings, particularly predialysis and immediate post-dialysis measurements. The 20-min post-dialysis BP best reflected interdialytic control. Extracellular volume measurements (ECF) using Bioimpedance spectroscopy (BIS) were reproducible, accurate, and confirmed the tight link between nutritional and volume status. The late increase in ECF and decline in nutritional status as end-stage approached, were only partially corrected by dialysis.

Relative blood volume (RBV) characteristics were investigated using short UF pulses (perturbation analysis), at different hydration states, to identify predictors of hypotension and approaching DW. RBV change was greater as predicted DW approached. The critical level of RBV reduction leading to hypotension showed a wide inter-individual variation. The approach to linearity of RBV decay curve was a surrogate for plasma refill (PR) and predicted impending hypotension. The role of heat exchange on cardiovascular stability was also examined.

Indocyanine green emerged as a suitable tracer for determining PV repeatedly during HD. The method was employed simultaneously with BIS to demonstrate abnormal proportional compartmental fluid distribution and assess fluid shifts and PR during UF. A significant relationship was demonstrated between ECF change, PR and haemodynamic stability. The dissociation between simultaneous absolute and RBV measurements was explained by a changing Fcell ratio, suggesting significant intravascular shifts within the microcirculation during UF.

Objective monitoring has improved understanding of the pathophysiology of UF during HD. However, the main factors limiting fluid removal during HD are patient-specific responses to UF. Individualised prescriptions will require use of systems to characterise patient responses, and prompt appropriate adjustments. These studies may contribute.

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Finally I would like to thank all the subjects who agreed to take part in the research and Fresenius Medical care, Germany for their financial and equipment support during some of the experiments.

LIST OF ABBREVIATIONS

AH Antihypertensive drugs

ABPM Ambulatory blood pressure monitoring

ACEI Angiotensin converting enzyme inhibitors

BV Blood volume

BSA Body surface area

BVM Blood volume monitor

BTM Blood temperature module

BMI Body mass index

CrCl Creatinine clearance

CRF Chronic renal failure

CO Cardiac output

CV Coefficient of variation

CHD Conventional haemodialysis

DPI Dietary protein intake

ECF Extracellular fluid

ECW Extracellular water

ESRF End stage renal failure

ESRD End stage renal disease

ECC Extracorporeal circuit

EDW estimated dry weight

GFR Glomerular filtration rate

HD Haemodialysis

HHD Home haemodialysis

ICG Indocyanine green

ICF = intracellular fluid volume

ICW Intracellular water

IBW Ideal body weight
IDH Intradialytic hypotension
 K_{ur} Ultrafiltration coefficient
LHD Long hour's haemodialysis
MAP mean arterial pressure
 PO_2 Partial pressure Oxygen
PCR Protein catabolic rate
PV Plasma volume
 P_v Pressure in blood compartment of dialyser
 P_d Pressure in dialysate compartment of dialyser
 Q_d Dialysate flow rate
 Q_b Blood flow rate
RRT Renal replacement therapy
RBV Relative blood volume
RAS Renin Angiotensin system
SBP Systolic Blood pressure
SVR Systemic vascular resistance
SD standard deviation
SV Stroke volume
SND Sympathetic nerve discharge
SNS Sympathetic nervous system
TMP transmembrane pressure
TPC Total protein concentration
TBW Total body water
URR Urea reduction ratio
UF Ultrafiltration
UFR Ultrafiltration rate
V = volume of distribution

INTRODUCTION

Chapter 1 *End stage Renal failure*

The principal functions of the kidneys are excretion of potentially toxic metabolites, volume and osmoregulation, maintaining acid base homeostasis and hormonal functions related to erythropoietin, 1,25 dihydroxycholecalciferol and renin. Progressive loss of kidney function is often described as chronic renal insufficiency in the early stages, chronic renal failure (CRF) when it is obvious and end stage renal failure (ESRF) when the disease reaches a terminal stage. The majority of patients with chronic renal disease progress relentlessly to ESRF.

The true prevalence of CRF is not known because many patients are unrecognised and not referred. Estimates have been made of 2000 p.m.p based on the prevalence of abnormal serum creatinine measurements in the population. Diabetes is a leading cause of ESRF (approximately 38 and 14 percent of newly diagnosed cases of ESRF are caused by diabetes in the US and UK respectively). Other causes of ESRF include hypertension, glomerulonephritis, primary and secondary glomerulopathies, cystic and interstitial renal diseases, and obstructive uropathy. (Table 1)

Symptoms and signs of renal failure tend to manifest quite late in the disease and can vary between nonspecific tiredness, anaemia, breathlessness, hypertension and generalised accumulation of excess body fluid. The major consequences of severe renal failure include cardiovascular disease, hypertension, sodium and water retention, anaemia, bone disease, hyperkalemia, metabolic acidosis and malnutrition.

One of the earliest effects of chronic renal disease is the power of the kidneys to compensate for changes in sodium and water intake. At GFR above 10 ml/min, patients with CRF but otherwise in good health maintain salt and water balance by a large increase in the proportion of filtered water and salt by magnification of the tubular response or fractional excretion of sodium. This capacity to handle water load is further impaired during procedures like surgery when factors such as nonosmotic vasopressin release operate and with in approaching end stage renal failure with loss of residual renal function.

1a Prevalence and treatment of ESRF

Individuals with end stage renal failure (ESRF) untreated will usually die within a few weeks. The life saving treatment options for renal replacement therapies (RRT) are either renal transplantation or dialysis (peritoneal dialysis or haemodialysis). The aim of RRT should be to restore patients to as normal a level of health as possible and to ensure full social and physical rehabilitation.

A well-functioning renal transplant is regarded as the optimal therapy of end-stage renal failure. Imbalance between demand and supply of cadaver organs, increase in waiting lists despite rigorous selection criteria and the increasing numbers of elderly patients with non-renal co-morbidity has created an ever-increasing population of patients who remain permanently on dialysis. In many units, this group constitutes well over 50% of the dialysis population. The sole future of these patients with end stage renal failure is that of a dialysis patient.

i) Prevalence of ESRF

End-stage renal disease creates a large burden for both individuals and society as a whole. The incidence rates in the United Kingdom, as well as in every other country, are rising steadily and continue to rise. UK is now treating over 30000 patients with end stage renal failure, at a rate of 566 per million populations. Approximately 53% of these are on dialysis. [1] The acceptance rate for new adult patients to dialysis is 96 per million populations. The annual increase in prevalent RRT patients is 4%. The estimated RRT population is over 45000 patients by 2010. This trend is being seen elsewhere (US over 250 new cases per million per year).

ii) Age, comorbidity and ESRF incidence

The prevalence of CRF rises with age and is higher in certain ethnic groups. The epidemic of renal disease and therefore patient acceptance for dialysis is particularly rising in the elderly. The increasing trend in incidence is much steeper for over 65 than in the younger age groups. (Fig 1) The median age of all patients on RRT in UK is approximately 55.9 years. This is 64.5, 58.3, 49.6 years for HD, PD and transplant

patients respectively. The median age of prevalent patients on HD has increased over the past 4 years [1]

In England and Wales 44% patients are over 65 and 1 in 6 over 75 years of age at the start of renal replacement therapy. The acceptance rate peak in those aged between 65 and 74. The UK Renal Registry data suggest that there is an unmet need in the 65+-age range. Presence of non-renal comorbidity in patients of ESRF is on the increase. This is not surprising when seen in the context of rising elderly population and liberal acceptance programs. Fig 2 compares two incident cohorts from the USRDS from 1986 and 1996 for both Peritoneal and Haemodialysis showing a dramatic rise in comorbidity. [2]

iii) Treatment of ESRF

Dialysis remains the predominant mode of renal replacement therapy in patients with ESRF. This can be delivered by either peritoneal dialysis or haemodialysis, both of which utilise the same fundamental principles of solute clearance and fluid removal across a semipermeable membrane but differ in their apparatus and technology.

Peritoneal dialysis (PD)

More than 22 years after its inception as an accepted modality of renal replacement therapy, the acceptance of PD varies across the world from a 40% usage in UK and New Zealand to 75% in Mexico. In the initial years PD is probably as effective as haemodialysis and is acceptable to many patients. It has certain social, logistical and medical advantages especially early in the course of RRT. Whether PD has a major or minor role in later years is unclear. Hospitalisation, long term technique survival and mortality is however not as good as in HD. USRDS data in the late 1980s have also suggested a worse prognosis for elderly and diabetics on peritoneal dialysis which constitute a significant majority of patients with ESRF.

Haemodialysis (HD)

At its inception, chronic haemodialysis was viewed as a therapy applicable to young adults with primary kidney disease and no substantive malfunction of other organ systems e.g. diabetics and elderly were excluded from dialysis therapy. By the 1990s

however the number of ESRF had risen beyond all expectations. The rising trend of treated ESRF can be attributed to an increase in incidence of ESRF as well as to a more liberalised acceptance of patients into dialysis programs. The rise in prevalence of ESRF has to be met in future with substantial increase in HD, especially in the elderly. In parallel with this growth, there has been considerable diversification both in the available technologies and the methods for provision. Haemodialysis can be "centre-based" in units located in hospitals or in free-standing facilities in cities, towns or rural areas. A comorbidity gap seems to be developing between PD and haemodialysis. Sicker, older, frailer patients seem to be directed preferentially to haemodialysis. The predominant form of dialysis at all ages is haemodialysis (Fig 3) and especially in the older age groups with a steep rise in prevalence of hospital based haemodialysis compared to other modalities.

1b Haemodialysis – historical aspects

The history of dialysis, only 40 years old, owes its invention to Thomas Graham (1805-1869), the father of modern dialysis, who laid the foundation of what later became colloid chemistry and demonstrated that vegetable parchment acted as a semi-permeable membrane. Parchment was coated with albumin to close defects, stretched over a wooden hoop and floated on water. Into this was placed, as on a sieve, a fluid containing crystalloids and colloids. He found that only the crystalloid material diffused through the parchment into the water. This phenomenon was termed "*dialysis*".

The artificial kidney

The next step, removing solutes through a semi-permeable membrane from the blood of an animal, was undertaken in 1913 by John Abel and his colleagues. Their dialyser, for which they coined the name *artificial kidney*, had a series of fragile and delicate celloidin tubes fastened by tying with a string to a system of glass manifolds. Thus, the flow of blood took place horizontally twice in each direction through 8 tubes in parallel. The capacity of their dialyser was limited and insufficient for human application. Hirudin, which they obtained by grinding up heads of leeches in solution, seemed to be toxic for human beings and was difficult to handle.

25 years later Pim Kolff started his experiments which led to the construction of an artificial kidney that was suitable for human application. The two main problems, anticoagulation and a suitable membrane, remained major obstacles. In 1914, V. Hess and McGuigan used Abel's vividiffusion method in a series of experiments on the dog improved the apparatus by creating a pulsatile bloodflow through the celloidin tubes, which apparently inhibited clotting. They also enhanced the procedure by mixing the dialysate at short intervals or continuously, preventing formation of a stagnant layer of fluid around the dialysing tubes.

First human dialysis

The credit for the first human dialysis must go to Georg Haas from Gieszen, Germany. [3,4] He circulated the blood of his animals through reed stalks, which apparently worked as dialysis membranes. On 18th February 1926, Haas finally attempted dialysis in a 20-year-old girl in the terminal stage of uraemia using hirudin and a continuous circulation technique. Haas later stated that his first attempts at a "Blutauswaschung" in a human during World War 1 in 1915 and 1916. Two years elapsed before Haas again tried the new haemodialysis procedure for therapeutic purposes: in 1928 he reported on two cases on 13th January 1928. In the second case, after dialysing 500 ml of blood through a 2.1 m² celloidin dialyser, 2.5 g of nonprotein nitrogen was removed with a gratifying symptomatic improvement. Blood pressure decreased from 205/110 to 145/95 mm Hg. Haas noted a decrease in the volumes of the blood during the extracorporeal circulation. Actually, a loss of 100 ml from the 500 ml aliquot occurred during 30 min of dialysis. Haas discussed the explanation of this phenomenon, which actually was caused by ultrafiltration, not by osmotic water removal because the dialysate was isotonic Ringer solution. Then followed a nine-year interval of silence on the subject during which time, two important advances were subsequently made: firstly heparin was purified and became available for human application and secondly a new cellulose product named cellophane was marketed, mainly for commercial use.

Dialysers

KOLFF'S Rotating Drum Dialyser

In the late 1930's, Willem ("Pim") Johan Kolff in Netherlands, had to treat a uraemic 22-year-old farmer's son, who subsequently died. Kolff began to think about an apparatus that could replace renal function and eventually he constructed a dialyser with large surface area, assisted by an engineer and a managing director of an enamel factory. It consisted of a cylindrical drum with a 40m length of sausage tubing wound around it to be perfused with the patients blood through a rotating coupling which according to Kolff was copied from a Ford automobile waterpump. Kolff's rotating dialyser was the first model that in practice was suitable for human application and made clinicians interested in the treatment of uremia.

Soon after Kolff's first series of publications other dialysers were constructed, all based on the same principles and using cellophane tubing. Nils Alwal, in Lund, Sweden, was the first to design a stationary coil type dialyser. This dialyser had the advantage that a *controllable ultrafiltration* could be achieved. [5]

Repeated access to the circulation and lack of a suitable dialysis technique for chronic renal failure proved a major stumbling block until in 1960, Dillard and Schribner reported on a bypass device made of Teflon which made the application of repeated haemodialysis in chronic renal failure a possibility. At the same time, Scribner and co-workers presented a new technique for circulatory access by arteriovenous cannulation which allowed repeated dialysis in a single patient. Scribner used modified Kill dialyser with a low volume in the blood compartment that could be primed by the patient at the start of dialysis with a washback using saline at the end.

Emphasis on various body spaces and disequilibrium

Claude Bernard was the first to point out that the kidneys don't have simply the role of excreting poisons but regulating our interior milieu. In 1923 Haas first observed that dialysis could remove volume from patients body, 20 yrs later Alwall designed the first dialyser with a *specific UF device*. Overhydration as part of uraemic toxicity had always been the interest of Alwall. He was the first to emphasise uraemic fluid

overload and became concerned about what he termed "uraemic fluid-retention, lung." He also suggested that dialysis not only detoxified but also dehydrated -- one of his biggest contributions. This was the first use of the term ultra-filtration and overhydration added to the spectrum added to the spectrum of uraemic toxicity. Alwall performed the first hemofiltration treatment and was the first to describe sequential ultrafiltration, which was later brought into clinical practice by Jonas Bergström.

The first chronic patients

On 9th March 1960, a Teflon arterio-venous cannula system was inserted in the arm of Clyde Shields, a 39-year-old machinist in terminal renal failure and on 23rd March, another pair of cannulae was placed in the right arm of Harvey Gentry, a 23-year-old shoe sales-man.

The results of repeated haemodialyses in these first two patients were surprisingly good: both became successful chronic dialysis patients. The system appeared to be so safe and reliable that patients with chronic uraemia could be haemodialysed entirely by nurses. [6,7] In April 1962, the Seattle group reported on eight patients who were on the treatment programme for intervals of four months and two years. Others, using somewhat different techniques, had more limited success but it soon became apparent that successful replacement of renal function and rehabilitation by regular haemodialysis in cases of irreversible, terminal renal failure had become a reality.

In 1966, a surgically created arterio-venous (A-V) fistula was introduced for access to the circulation by Brescia, Cimino, Appel and Hurwich another landmark in the history of dialysis. [8]

Central dialysate supply system

The introduction of the single pass technique (dialysate was pumped from the tank, heated to 37C and discarded after a single pass through the dialyser) and substitution of acetate for bicarbonate; in the dialysis fluid [9] led to the development of a centralised dialysate fluid supply system. A central multipatient system, a concentrated solution of salts and dextrose had to be diluted with (pre-treated) tapwater by means of an accurate proportioning system for a continuous supply of

dialysis fluid. Sodium bicarbonate unlike sodium acetate could not be mixed with concentrated calcium and magnesium salts without adjusting pH to 7.4 or less.

The Start of regular dialysis

In August 1965, some 160 chronic patients were on treatment in Europe and 40 centres had started treating patients with chronic renal failure. Soon, the number of chronic patients requiring treatment outnumbered the available facilities, both in Europe and in the United States.

Home dialysis

Despite a gradual increase in treatment facilities, enormous problems of financing and training of doctors and nurses had to be faced and in an effort to achieve at least a partial solution of the dilemma Shaldon and co-workers of the Royal Free Hospital in London introduced the concept of self dialysis in September 1964. [10] From self dialysis in the hospital to home dialysis was the next step.

The Seattle group started their home program in September 1964. The Royal Free team began home dialysis two months later, their first patient being a registered nurse. However through the years with acceptance of more complicated and elderly patients, advent of peritoneal dialysis and other reasons, home haemodialysis numbers have decreased dramatically.

Basic research on haemodialysis focused on haemodynamics, kinetics of solute removal and development of new membranes. After 1965, industry became increasingly interested in dialysis and a growing number of proportioning machines, monitoring equipment, blood pumps and ancillary equipment was constructed and introduced. A plethora of types of dialysers was made available, the performance of which was further improved by replacement of the cellophane with thin Cuprophan membrane.

Around 1965, a new design, the hollow fibre artificial kidney, or capillary dialyser was introduced. [11,12] With a short length of the blood path, a thin blood film, highly permeable membranes and excellent convective capability, this improved dialyser could control uraemia very well.

The square metre/hour and middle molecule hypotheses

In the early years of regular peritoneal dialysis for chronic renal failure, Scribner noted despite a certain amount of 'underdialysis' with chronic peritoneal dialysis, peripheral neuropathy either did not occur or did not progress. Suspicion arose that the so called middle molecules played an important role in the toxicity of uraemia. Because of their size, it was suggested that they were very slowly removed compared to urea. The cellulose membranes used in haemodialysis (e.g. cellophane and Cuprophane) have a high membrane diffusion resistance for these species of molecules.

This speculation correlated with the hypothesis that using a given membrane, prevention of peripheral neuropathy depended on a minimum number of hours of dialysis per week, rather than on maintaining specified levels of blood urea and creatinine. Further, it was suggested that larger toxic solutes must permeate the peritoneum better than cellulose membranes. These considerations in relation to the adequacy of dialysis led to the square metre/hour hypothesis [13,14] and to its modification, the middle molecule hypothesis. [15] Both theories suggest that inadequate removal of the middle molecules (molecular weight between 300 and 2000 daltons) cause complications such as peripheral neuropathy, pericarditis and perhaps others.

Since the removal rate of middle molecules is slow, the diffusion gradient remains high throughout dialysis, unlike urea. Thus, the net removal rate of middle molecules remains rather constant during protracted dialysis and net removal is proportional to the total number of haemodialysis hours/week unlike urea, which has flow dependent removal, which decreases with the decreasing plasma concentration as dialysis proceeds.

Short dialysis

The square metre/hour and middle molecule hypotheses have profoundly influenced dialysis strategies in recent years. The array of protocols used resulted in conflicting clinical results. [16,17] In many dialysis centres, protocols were changed according to the new theories, resulting in short dialysis schedules either with large surface area dialysers or with the same type of dialyser and increased dialysis frequency.

Sophisticated modifications of dialysis protocols have used a special membrane with high clearances for molecules in the critical range of 300-2000 molecular weight. Since Wolff described the 8 cases of uremia treated by HD and UF, extracorporeal technology has advanced and has been broadly applied throughout the world in more than 1 million patients who have developed acute and chronic renal failure. The proportional distribution of these patients across the globe has been heterogeneous. (Fig 4)

1c Dialysis Adequacy and Mortality

When the euphoria of the first years after introduction of dialysis treatment was over, it became evident that mortality remained unacceptably high and life expectancy with ESRF is poor despite renal replacement therapy. (Fig 5) Improvements have occurred in dialysis machines, in water-purification systems, and in the composition of dialysate and the performance and biocompatibility of dialysers. Despite these improvements, mortality and morbidity among patients treated with dialysis, particularly mortality related to cardiovascular diseases and morbidity resulting from complications of vascular and peritoneal access, continue to be unacceptably high.

i) Mortality among Patients on Dialysis

The dialysis outcomes and practice patterns study (DOPPS) in an analysis of patient survival in UK, noted a significantly higher mortality rate compared to some other European countries. The adjusted risk of mortality in haemodialysis patients in the UK is 36% higher than in four other European countries participating in DOPPS. [18]

Deaths in haemodialysis subjects are due mainly to cardiovascular diseases and infections (approximately 50 percent and 15 percent of deaths, respectively). Hypertension continues to be a major risk factor for cardiovascular disease; recent surveys show that more than 50 percent of patients undergoing outpatient haemodialysis have a systolic blood pressure before dialysis of more than 150 mm

Hg [19] Other common risk factors for cardiovascular disease includes depressed high-density lipoprotein cholesterol levels, coronary-artery calcification, diabetes, and left ventricular hypertrophy.

Malnutrition has been estimated to be present in up to 50 percent of patients with ESRD and to be independently associated with increased morbidity and mortality. [20]

ii) Adequate Haemodialysis

Inadequate haemodialysis not only shortens survival but leads to anemia, malnutrition, functional impairment and frequent hospitalisations that increase health care costs. Because inadequate HD is often undetected unless it is severe and prolonged, it is important monitor adequacy indices.

The large differences in treatment outcome between centres and countries strongly suggested that these were related to the way treatment was practised. In 1975 the National Institute of Health decided to investigate this problem. This resulted in the concept of urea kinetic modelling that showed not only insufficient dialysis but also low urea levels due to inadequate nutrition and low protein catabolic rate were associated with increased mortality [21]. Subsequent analysis suggested that the effectiveness of the treatment should be expressed in the Kt/V formulae [22]. In this concept, Kt/V is a dimensionless index based on the urea clearance rate, K , and the size of the urea pool, represented as the urea-distribution volume, V . K , the sum of clearance by the dialyser plus renal clearance, is multiplied by the time spent on dialysis, t . In short the formula represented total urea clearance standardised by body volume. Measurement of the "delivered dose" of dialysis has therefore focused on the removal of urea, an easily measured surrogate marker for uremic toxins. The quantity of urea removed during HD is a clinically accepted surrogate to define the patients risk profile. The other commonly accepted measure of HD dose is urea reduction ratio (URR), based on fractional reduction of blood urea concentration during a single dialysis treatment, calculated by dividing the decrease in urea (predialysis – postdialysis)/ predialysis concentration expressed as a percentage. Factors that may adversely affect the adequacy of dialysis include recirculation between venous and arterial limbs in the dialysis catheter, inadequate blood or dialysate flow rates, poor-

quality reprocessing of the dialyser, and skipping or early termination of dialysis treatments. The most frequent cause of premature termination of dialysis was found to be hypovolemic symptoms of hypotension, cramps and nausea.

There is overwhelming evidence that the amount of delivered haemodialysis is an important modifiable predictor of death in HD patients. Patient mortality is higher when the amount of urea removed is less. The relationship between URR and mortality is nonlinear. Retrospective studies of mortality outcome for patients with ESRD suggest that odds of death progressively increase when URR falls lower than 60% to 65% [23]. Such findings and an evidence based professional consensus led to national organisations in the UK (Renal Association) and USA to advocate a minimum URR of 65% or Kt/V 1.2 as thresholds for adequate HD [1]. Unfortunately, based on these standards many patients still have a suboptimal dialysis dose. [24] The use of modern membranes made it possible to achieve a high Kt/V (surrogate for "adequate dialysis") within a shorter time. Increasing burden of ESRF, dialysis economics favoured the use of shorter dialysis time. Such a policy however has made volume control difficult and often dialysis unpleasant with the use of more cardiovascular medications.

iii) Optimal dialysis

The relationship between dose of dialysis and outcome remain complex. Adequate dialysis represents a treatment that rehabilitates the patient, minimises treatment related side effects and corrects biochemical abnormalities associated with loss of renal function. What constitutes an "optimal" dose of dialysis, above which no further improvement in survival or well being can be achieved, is not known. Retrospective data in one study showed that survival rates do not rise as the urea-reduction ratio rises above 70 percent, or as Kt/V rises above 1.3. [25] In a more recent study, [26] patients treated with URR >75% had a substantially lower relative risk than patients treated with URR 70 to 75% ($P < 0.005$ each, for medium and small BMI groups). It was concluded that a higher dialysis dose, substantially above the Dialysis Outcomes Quality Initiative guidelines (URR >65%), is a strong predictor of lower patient mortality for patients in all body-size groups. The HEMO Study was a controlled trial of dialysis dose and membrane flux in 1846 haemodialysis patients followed up for 6.6 years in 15 centres throughout the United

States. [27] The study attempted to determine whether the type of dialysis membrane used in haemodialysis or the amount of dialysis (measured by Kt/V) that is prescribed could influence well-being and survival. The data suggest that mortality and morbidity might be reduced by increasing the dialysis dose above the current standard in women but not in men. This effect was not explained by differences between men and women in age, race, or in several indices of body size.

The HEMO study measured dialysis therapy using the Kt/V index and focussed on urea as the surrogate uremic toxin. Efforts to improve disappointing mortality figures have been unfortunately concentrated on urea removal, neglecting BP and volume control and other important factors. The replacement of the kidneys task of regulating body fluids, circulation and blood pressure, bone metabolism and nutrition are not included in the current measures of adequacy. Yet the major cause of morbidity and mortality is cardiovascular complications that are closely related to correction of circulatory disturbances.

Chapter 2

Cardiovascular complications of dialysis

Cardiovascular disease is much more common and widely prevalent in the ESRF population compared with the general population (Fig 6) and accounts for nearly 50 percent of all deaths in patients on haemodialysis. There is an increased incidence of congestive cardiac failure, ischemic heart disease and myocardial infarction among patients with end stage renal disease attributable to hypertension, anemia, hyperlipidemia, hyperhomocysteinemia, accelerated atherosclerosis, and possibly impaired oxygen delivery to the myocardium induced by uremic toxins.

2a Hypertension in Haemodialysis

While hypertension occurs in approximately 15% of the general population, its frequency is much higher in patients with renal disease. The incidence increases in parallel with progression of renal failure, and approximately 80- 90 % patients with progressive renal failure approaching maintenance dialysis have hypertension. Systolic hypertension with or without diastolic hypertension is a major problem in haemodialysis (HD) patients; isolated diastolic hypertension is uncommon. Accelerated age-related changes in vascular stiffness, together with factors peculiar to uraemia, lead to loss of large and small vessel distensibility and profound changes in circulatory function that includes an increase in systolic pressure and widening of the pulse pressure. The appearance of hypertension is related to the nature of the renal disease. It is most frequent - and may start before functional impairment - in various glomerular diseases and polycystic kidneys, while its onset is later in primary tubular and interstitial disorders. This difference is probably due to differences in salt excretion impairment and/or in activity of the renin-angiotensin system. Once the patient has become anuric, the relation of BP to the underlying disease is less clear, but this subject has not been sufficiently analysed. When dialysis treatment is started and excess of body fluids is removed by UF, hypertension disappears in many patients during the first months.

Available reports show large regional differences in prevalence of hypertension in dialysis patients. In one Italian centre, hypertension decreased gradually from 80% to 40% of the patients during the first half year of treatment. In an EDTA questionnaire survey in 1992: Pre-dialysis systolic BP was greater than 140 mmHg in 70% of the patients and diastolic pressure above 90 mmHg in 30% haemodialysis patients. The median BP was 145/85. In 1994 the Health Care Financing Administration reported a mean BP of 152/79 mmHg in the USA. Based on an in-depth review of the literature the HCFA committee could not find 'enough data to submit an evidence-based clinical practice guideline'. Another study (28) found that blood pressures greater than 160/90 in 62% of the patients and conclude hypertension is not adequately controlled. These figures are even more striking if one considers that the majority of the patients were treated with antihypertensive drugs. Recent UK registry data reports no significant improvement in BP adequacy in HD over the past few years. [24] In sharp contrast, several other reports have much better BP values. These were from centres where much attention was given to volume control and salt restriction.

The failure to achieve BP control by the great majority of dialysis centres the world has led to speculation that the present-day patients case-mix is different from the past and also varies between populations. However in one centre [29] 72% of the patients had normal BP without drugs (MAP 89 ± 12 mmHg) the others had a mean MAP of 97 ± 9 mmHg. A Turkish centre reported a mean BP of $131/80 \pm 17/9$ mmHg in all 67 patients treated with volume control during one year [30] The most impressive results were obtained in Tassin [31] where BP could be normalised in 97% of the 712 patients (Charra et al. 1992). An important element of dialysis treatment in these centres is strictly restricted sodium intake and better volume control with intensive ultrafiltration sessions. It is probable that these factors contribute to better outcomes.

Hypertension and mortality

Although relationship between BP and mortality in dialysis patients is complicated there is now strong evidence that hypertension is a significant factor in late mortality.[32] (Fig 7) Previously, when no effective treatment of hypertension was

available, the conviction prevailed that high blood pressure was inescapable and that lowering it might even be harmful. Large trials have shown such significant decreases in morbidity and mortality after antihypertensive treatment that this may be considered one of the most important successes of modern medicine. In dialysis patients' hypertension was likewise considered to be a risk factor for their high cardiovascular morbidity and mortality.

Indeed, hypertension is a major contributor to well-established risk factors such as increases in left ventricular mass index and volume as well as atherosclerosis, ischemic heart disease and de novo cardiac failure in dialysis patients. Therefore it is likely that hypertension itself is involved in the development of these complications. There is a considerable body of epidemiological data suggesting a relationship between hypertension and mortality in dialysis. But there is also data suggesting the lack of a relationship between blood pressure and mortality, and even that lower predialysis blood pressure may increase mortality risk. In one large study from the USA only systolic BP > 180 was associated with decreased survival. It was found that low blood pressure has a particularly bad prognosis [32] (Fig 8). A recent survey of 649 HD patients found an inverse relationship with survival over the entire BP range: patients with MAP > 115 mmHg had 56% longer survival than those with MAP > 101 mmHg. [19] Others have also concluded that the association between predialysis hypertension and mortality was surprisingly small [33].

In sharp contrast, a group of investigators [34], who applied meticulous volume control with long dialysis sessions, not only achieved better BP control than almost all other centres, but also had better survival figures, and found a significant reduction in death rates with lower BP values even for levels within the normal range. It is well known that heart failure causes a decrease in blood pressure. The 'J' or 'U' curve seen in elderly patients with essential hypertension can be explained by the fact that the previously high BP decreases before the patient dies from heart failure. Unfortunately, most multi-centre studies that suggest that hypertension is not harmful in dialysis patients do not provide information on the condition of the heart. However, the authors of one study [35] suggest that a similar explanation of this paradox applies to dialysis patients: BP fell when heart failure developed, and heart failure pre-dated most deaths in their study.

Similar data from other group of investigators confirm a U-shaped relationship between blood pressure and relative death risk in dialysis patients for systolic and diastolic values. [36] Therefore both elevated and low blood pressures are potential risk factors in the dialysis population.

Optimal blood pressure control (achieving normotension and avoiding hypotension) is an essential prerequisite to patient longevity. This is of importance in patients treated with dialysis who are at a very high cardiovascular morbidity. Hypertension is of greater prognostic significance in the elderly who form a major group in present dialysis population because of their markedly higher background risk. In the normal population studies such as the HOT study [37] show that the benefits from treating the elderly are similar to that in young patients. The British Hypertension Society, WHO guidelines adopt the same treatment thresholds and targets for the elderly as for younger patients, stressing the need for such accurate BP control in an average dialysis patient. The standards set for BP control have become more difficult to achieve in dialysis patients [24].

Some problems in the treatment of hypertension in dialysis arise perhaps with the diagnosis itself of hypertension in HD patients. Among nephrologists, owing to the intermittent nature of the treatment, there is still debate concerning the appropriate target blood pressure for patients because of the intermittent nature of haemodialysis and UF.

2b Hypertension in haemodialysis: The role of volume overload

The first patient surviving on chronic haemodialysis (1960) suffered from malignant, seemingly intractable hypertension, but became normotensive after aggressive ultrafiltration. B. Scribner then stated, "As in the case of nephrectomized dogs, hypertension appears to be influenced by the size of the extracellular space. The combination of dietary sodium restriction and ultrafiltration during dialysis permits regulation of the extracellular volume." [38]

One of the most harmful consequences of uncontrolled uraemia is the excess saline retained. Fluid retention during progressive renal failure leads to the almost universal presence of hypertension at the initiation of dialysis. This aggravates hypertension and increases the left ventricular load.

Several studies have shown that expanded blood volume and extracellular fluid volume is increased in early and late renal failure and that this correlates to elevated blood pressure. [39] Fluid overload, by increasing venous return to the heart, may contribute to the increased cardiac output observed in patients with renal failure. Some degree of extracellular volume expansion is present in the majority of patients with renal failure and may contribute to the apparent well being of the patients despite clinical and biochemical evidence of malnutrition. The well-documented post transplant diuresis is attributed to extracellular volume expansion in the nonoedematous patient.

BP control – what can be achieved

Thompson et al followed by several others in the 1970's reported achieving normotension in 90% dialysis patients. [40] Different groups in France (Tassin) [41], and New Zealand (Christchurch) [42], using long dialysis, have reported excellent drug-free BP control, as was the case for 90% of patients on dialysis in the early 1970s. Tassin has reported excellent BP control with meticulous salt and volume removal, long dialysis and with minimal use of antihypertensives. These results have also been reported from other units using conventional dialysis (normotension 70 –

96%) with aggressive and often extra UF sessions. [43] Table 2 The quoted incidence of dialysis resistant hypertension in the 1970s varied from less than 5% to more than 30%. The percentage of dialysis patients quoted to have dialysis resistant hypertension and therefore antihypertensive medications varied considerably depending on the approach of the specific dialysis unit and of the individual physician. Demographic differences, differences in the spectrum of renal disease, variable ultrafiltration policies, differences in defining hypertension in dialysed patients and ease of acceptance of medication by the patient population may have all contributed to the reported differences. Figure 9 illustrates this in a state in the USA where nearly 70% patients have been reported as hypertensive despite a large majority of them being on antihypertensives. The high prevalence of dialysis hypertension despite the extensive use of antihypertensives suggests that antihypertensives alone are ineffective.

2c Other Mechanisms of Hypertension (Table 3)

i) Renin angiotensin mechanism (RAS)

The RAS plays an important role in the regulation of BP in normal man and in some hypertensive patients. Renin secretion by the non-functioning kidney in dialysis patients is not completely abolished and sometimes may be inappropriately high. In such cases, hypertension is resistant to volume control but may be lowered by bilateral nephrectomy or renin lowering drugs, in particular angiotensin antagonists or converting enzyme inhibitors (ACEI). Unfortunately, hardly any systematic, long- term investigation has been performed on this subject and as a result contradicting conclusions have been drawn which are difficult to reconcile. Because renin acts as a hormone and is subject to feedback regulation in normal man, it is plausible to assume that this regulation must be disturbed when normal function and structure of the regulating organ is completely lost. Indeed available reports indicate that changes in plasma renin activity (PRA) which occur in normal persons upon changes in posture and volume are absent or diminished in dialysis patients. In particular, PRA is not influenced by the dialysis procedure. However, not unexpectedly there seem to be differences in sensitivity of the renin secretion to UF

between individual patients. [44] Several reports have indicated that PRA is too high in relation to the volume status of hypertensive patients and that BP cannot be related to the product of (log) PRA and volume. However, others could not confirm this contention. While some authors consider that the contribution of the RAS to BP is minor; others conclude that it plays an important role in nearly every dialysis patient. The latter view is strengthened by the fact that bilateral nephrectomy decreases BP in most patients including those whose PRA is not elevated. However the influence of RAS seems to be minor when overhydration is present. Part of the divergence of views may be due to the large interindividual variability. Vascular reactivity to vasoconstrictive stimuli is often diminished in dialysis patients. Besides 'volume' and 'renin', several other abnormalities have been detected in dialysis patients and thought to have a role in the genesis of hypertension.

ii) Sympathetic system

As in essential hypertension, much effort has been expended looking for signs of increased sympathetic activity in dialysis patients. Most tests, in particular measurement of plasma catecholamines, have yielded negative or equivocal results. However, the large decrease in BP observed after pharmacological sympathetic blockade has provided indirect evidence for a role of this system. This was confirmed by the use of a new technique: direct measurement of sympathetic nerve discharge (SND). Converse et al. found that SND in HD patients was 2.5 times higher than in healthy control subjects. Remarkably, bilateral nephrectomy was associated with normalisation of sympathetic tone. Thus sympathetic activation seemed to be mediated by an afferent signal arising in the diseased kidney. SND was unrelated to plasma norepinephrine levels, confirming that they constitute an inadequate tool to measure sympathetic activity. There was also no correlation between SND rate and PRA. On the other hand, Ligtenberg et al. [46] showed that chronic (but not acute) ACE inhibition decreased SND together with BP in CRF patients not on dialysis treatment. Cardiac congestion is also accompanied by increased SND, which decreases with treatment. Some of Converse's patients had severe hypertension and may well have been overhydrated. It cannot yet be

concluded with certainty the degree to which increased SND contributes to hypertension, if at all.

iii) Circulating substances

Elevated levels of blood-pressure-increasing factors have been detected in the blood of HD patients and implicated as causes of their elevated BP levels. Among these are endogenous digitalis-like substances, endothelin-1 and asymmetric dimethyl arginine (ADMA). [47,48] The latter is an inhibitor of the synthesis of a potent local vasodilator nitric oxide (NO) and is insufficiently cleared by dialysis. Insufficient production of vasodilator factors like prostacyclin has also been implicated. [49,50]

There are a number of factors, which also operate in the normal population, such as cardiac status, vascular elasticity, rigidity and contractility, autonomous nervous function, catecholamines, the renin-angiotensin system, PTH, nitric oxide, endothelin, erythropoietin and of course antihypertensive drugs.

iv) Paradoxical hypertension

When volume is reduced by ultrafiltration during dialysis, the most frequent complication is an excessive decrease in BP. In some patients, however BP rises despite fluid removal. This has often been described as paradoxical hypertension.

This is not reversed by ACE inhibition making it less likely to be renin mediated. In some studies with repeated UF their BP decreased, intradialytic rises subsided and the cardiac volumes improved. Hypervolemia therefore appears to be an important factor in this phenomenon. Several studies show that even so-called resistant and paradoxical hypertension in most cases are due to saline overload and are responsive to fluid extraction (51,52).

Even in units in which there has been a particular interest in hypertension in dialysis patients, 15-25% needs hypotensive drugs, and this may rise to 50% in other units. The higher figures suggest that there has been inadequate salt and water removal during HD and that patients have not controlled their intake sufficiently between dialysis sessions. The fact that even when this has apparently been achieved with dialysis duration 12hrs /wk, hypotensive drugs may be needed in a quarter of patients, while in Tassin (24hrs/wk dialysis) they are hardly ever used, raises the

possibility that longer hour may confer an as yet undefined advantage. The best evidence available in terms of a comparative study refutes this hypothesis. Recently, Swedish normotensive and hypertensive patients were compared with normotensive patients in Tassin using bioimpedance and vena cava ultrasound. [53] The results clearly showed that predialytic and postdialytic extracellular volume space was greater in hypertensive as compared to normotensive dialysis patients. The Tassin patients achieved adequate volume and salt removal by virtue of the long time available for this in each dialysis session, but it appears that it was possible to achieve in shorter times. The achievement of normovolemia is not in itself a reason for advocating dialysis sessions of 8 hours three times weekly. Indeed, in Parma, Cambi has obtained survival similar to that reported by the Tassin group using 12 h HD weekly. [54]

There is convincing evidence that hypervolemia is an important contributant to the large proportion of hypertensive dialysis patients. Although there is also convincing evidence that a host of vasoactive factors are involved in the pathogenesis of dialysis hypertension (nitric oxide synthase inhibitors, increased sympathetic outflow, RAS activation etc.) they appear to operate in relation to a degree of fluid excess. Hypertension adds a pressure load to the volume load on the heart and therefore prevention of fluid overload is essential. Removal of the excess fluid in a safe and regulated manner constitutes a primary goal in the reduction of an important cardiovascular risk factor in dialysis patients.

Chapter 3

Vascular instability syndromes

The most important outcome in HD is, of course, survival. Less emphasis has been placed on factors less easily measured e.g. morbidity associated with treatment (interventions, intradialytic morbidity, hospitalisations) or quality of life issues. Dialysis related complications contributes to significant morbidity and negatively affect patient outcome. The most frequent complications during HD are in descending order of frequency hypotension (20 – 40 %), cramps (5 – 20%), nausea and vomiting (5-15%), headache (5%), chest pain (2 – 5%), back pain (2 – 5%), itching (5%), and arrhythmias, hypoxemia fever and chills (less than 1%) and rarely anaphylactic reactions. The first three major complications (30 – 70%) are predominantly related to disturbances in fluid balance and overall blood volume regulation.

3a Intradialytic hypotension (IDH)

The realisation that a drop in blood pressure can adversely affect patient morbidity and mortality is not a new concept but its impact on outcome is less appreciated. A number of physiologic abnormalities and treatment related complications heighten the link between hypotension and adverse outcomes in ESRF.

Despite the fact that the quality of life of patients undergoing haemodialysis and the growing understanding of the pathophysiologic mechanisms that contribute to haemodynamic instability during haemodialysis has improved in the last 20 years, hemodialysis-associated hypotension still remains a significant cause of patient morbidity. Symptomatic hypotension is still one of the major and most frequent complications occurring during dialysis therapy. It is characterised by symptoms such as dizziness and nausea. The incidence of intradialytic hypotension is estimated at 25-30% [55] and is expected to increase because of an increasing number of elderly and cardiovascularly compromised dialysis patients. In these patients, structural cardiovascular abnormalities such as decreased left ventricular compliance,

systolic dysfunction, and decreased venous compliance are common. These structural cardiovascular changes have important clinical implications, making dialysis patients more susceptible to the development of intradialytic hypotension. The incidence of hypotension increases with age. (Table 4) In the United Kingdom now the average age of the haemodialysis population is over 60 yrs. [24]

Recent reports also suggest that patients with sustained hypotension are at a greater risk of death than chronically high BP readings. Zager et al in a study of more than 5000 HD patients followed up for a mean of 2.9 ± 1.8 years noted that 49% patients entered into the study died. [32] Using Cox regression analysis they reported a U shaped relationship between SBP and cardiovascular mortality. The relative death rate for patients with predialysis or postdialysis hypotension increased to 4 times normal or 2.5 times normal respectively. Tisler et al noted lower survival in patients with frequent intradialytic hypotension. [56] Considering the potential risks of low BP, IDH can no longer be treated as a benign condition.

Theoretically hypotension can contribute to an increased relative risk of death in ESRF by several mechanisms, which include acute coronary syndrome, autoregulation dysfunction, ischemia and arrhythmogenicity. Endothelial abnormalities (altered procoagulants, thrombogenicity and alterations in coronary flow reserve) and altered vascular distribution within the myocardium provide an environment for vascular injury. Despite the lack of hard evidence, it seems desirable to attempt to minimise the frequency of IDH episodes. There is evidence that life threatening conditions such as nonocclusive mesenteric ischemia are associated with frequent intradialytic hypotension and corollary damage to brain and cardiac tissue might also be expected. Another reason to avoid intradialytic hypotensive episodes is that they often result in underdialysis, because they often engender treatment interruptions, lowering of blood flow rate etc and when associated with symptoms, may be responsible for patients reluctance to extend dialysis time. Studies have also shown that IDH and hypovolemic symptoms are frequent reasons for premature termination of dialysis or missing dialysis sessions (Fig 10) ultimately leading to inadequate dialysis.

Intradialytic hypotension is characterised by an abrupt decline in blood pressure. Episodes may occur early (< 1hr), late (>1hr) or unremitting posttreatment. It may be abrupt, temporary or sustained. Identifiable risk factors for IDH include diabetes, left ventricular hypertrophy and diastolic dysfunction, CCF or previous MI, high

interdialytic weight gains (> 3% body weight) and the anephric state. Hospitalised patients are at a unique risk for IDH based on their underlying reason for hospitalisation, suboptimal metabolic status, cardiovascular status and perioperative condition. These clinical conditions may compromise a person's ability to respond to volume during dialysis. The ESRF population at risk of IHD continues to grow as age and number of comorbidities of incident patient increases every year.

The morbidity of IDH ranges from subtle complaints of not feeling well, through the mild to moderate of visual complaints, cramping and nausea to the more, serious, vascular complications that include cerebral infarction, cardiac ischemia, vascular access thrombosis, nonocclusive mesenteric ischemia and arrhythmias. IDH episodes may resolve with treatment in the unit or persist after the patient leaves the unit and returns home. IDH events not only carry an increased risk of end organ damage but also are disruptive to the unit and distressing to the patients.

3b Pathways to Hypotension (Fig 11)

i) Volume depletion

Dialysis hypotension occurs because a large volume of blood water and solutes are removed over a short period of time. These overwhelm compensatory mechanisms, including plasma refilling and reduced venous capacity. Symptomatic hypotension may occur as a result of a decrease in blood volume and reduced vascular reactivity during dialysis in combination with structural abnormalities of the cardiovascular system. Haemodialysis treatment requires the removal of fluids from the circulating blood to return the hydration state in the patients undergoing chronic treatment to normal. During HD (diffusion combined with ultrafiltration), the plasma water removal induces a progressive reduction in blood volume (BV). The haemoconcentration is followed by a fluid shift from the interstitial and cellular spaces toward the vascular compartment. Hypovolemia triggers the activation of the sympathetic system, which induces vasoconstriction and an increase in myocardial contractility and heart rate. The maintenance of blood pressure is related to two fundamental mechanisms: BV preservation and cardiovascular compensation. Arterial hypotension occurs when central hypovolemia causes an underfilling of the cardiac chambers, thereby compromising the circulatory load, while either the

vascular arteriolar or venous tone decreases or is inadequate in relation to the stroke volume reduction.

Studies have suggested that some patients whose volume is depleted by ultrafiltration exhibit a paradoxical withdrawal of efferent sympathetic tone. [57] This response represents a type of autonomic dysfunction. This dysfunction is caused by a distortion of the mechanoreceptors in the left ventricle, triggering a response that leads to a paradoxical sudden decrease in efferent sympathetic tone and eventually to profound hypotension. Although autonomic dysfunction related to volume depletion is probably far less common than the other type, it may be a significant factor in patients who undergo severe volume loss during dialysis.

ii) Decline in Plasma Osmolality and other mechanisms

Decreased plasma osmolality is another important cause of intradialytic hypotension. If the dialysate sodium is below 135 mEq/L, removal of ultrafiltrate during dialysis is achieved via a disproportionate depletion of the extracellular vs intracellular space. If the dialysate concentration is increased to greater than 140 Meq/L, more volume is recruited from the intracellular space during ultrafiltration, thereby preserving the integrity of the extracellular space and plasma volume. This technique has the net effect of sustaining blood pressure.

Acetate Dialysate Buffer

Acetate dialysate buffer has been shown to be associated with a greater number of untoward hemodynamic reactions as compared with bicarbonate dialysate buffer. These reactions are believed to be related to the vasodilatation that is caused by acetate. [58] Most dialysis units now use bicarbonate dialysate to circumvent this problem.

Limited Cardiac Reserve and Myocardial Ischemia

Several cardiac abnormalities may contribute to intradialytic hypotension. [59]

Overt or silent coronary ischemia, which occurs at a high rate in patients with ESRF, may impair myocardial reserve. During periods of stress, inadequate responses to the loss of plasma volume may ensue.

Left Ventricular Hypertrophy and Dysrhythmias

While congestive heart failure is estimated to occur in approximately 25% of patients with ESRD, the majority of this population show echocardiography evidence of left ventricular hypertrophy, which is another factor in impaired myocardial reserve. When plasma volume is reduced during dialysis, the inability of the left ventricle to sufficiently respond to this alteration leads to hypotension. Several types of left ventricular hypertrophy are present in patients with ESRF. Patients may exhibit either systolic dysfunction; characterised by low cardiac output and a widely dilated left ventricular cavity, or diastolic dysfunction, characterised by a relatively small left ventricular cavity and a normal ejection fraction. In diastolic dysfunction, the primary derangement is the inability of the left ventricle to relax during diastole. Another aspect of left ventricular dysfunction is that patients may not only have hypotension due to impaired myocardial reserve but also an inability to accept large volumes of fluid, especially if they have diastolic dysfunction. Arrhythmias may also occur during dialysis, particularly in patients with left ventricular hypertrophy and underlying myocardial ischemia. [60] Occasionally, arrhythmias may result in hypotension. Any medications that affect cardiac rhythm should be scrutinised if they are being administered during hypotensive episodes.

Hypoxemia

Another complication that is often seen during dialysis is hypoxemia. [61] Whether the usually mild hypoxemia that occurs during dialysis is capable of causing adverse events is still a source of debate. Arterial partial pressure of oxygen (PO_2) typically falls between 5-8 mm Hg during the first hour of dialysis. If bicarbonate dialysate buffer is being used, the subsequent decrease in arterial PO_2 may be attributed to a slight decrease in minute ventilation. In most patients, a mild reduction in PO_2 is not a significant problem. However, in patients with underlying chronic pulmonary disease and an already compromised PO_2 , dialysis may exacerbate the hypoxemia leading to untoward symptoms. This is usually treated by supplemental oxygen during dialysis.

Food ingestion

The ingestion of food during dialysis can cause splanchnic vasodilatation, which can be an etiologic cofactor in lowering blood pressure. [62]

Anaphylactic Reactions [63]

Anaphylactic reactions are classified into two main types. Type A has been described as the classic anaphylactic reaction that is essentially believed to be an allergic response to an allergen in the dialyser. Type B is less severe but more common than type A. The type B reaction is characterised by pain in the chest or back and usually occurs within the first few minutes to the first hour of dialysis. The aetiology of type B reactions is unknown.

Preventive measures include an accurate evaluation of dry body weight, the use of bicarbonate as buffer, and an adequate sodium concentration in the dialysate. However, the impairment of both myocardial and venous compliance can make the patient more susceptible to a volume contraction making these remedies only partially successful. Developing a preventive strategy or a tool, which has the response of the individual patient, as it would be an important contribution.

Chapter 4 *Concept of dry weight*

4a Definition of dry weight

Even though the concept of dependence of blood volume regulation in HD on the ECF appeared with maintenance dialysis itself, it was at least 7 yrs before Thomson et al [64] coined the term *dry weight* and defined it as achieving a state of normovolemia with hypotension not explained by other causes. Subsequently in 1970, even if they did not call it by that name, most authors used the dry weight concept and reported 90% dialysis pts achieved normotension. Blumberg confirmed at the same time that normotension at dry weight correspond to an ECF in the normal range. [65] 3 years later Kim showed that the presence of hypotension does not necessarily mean dry weight has been achieved. [66] Fishbane and others also reported that a limited proportion of patients resisted fluid or sodium removal. [67] Clinical wisdom suggests that dry weight is often not assessed accurately during routine HD therapy, and this notion is supported by the observation that hypertension is present in a significant fraction of chronic HD patients. Although the mechanisms leading to hypertension are multifactorial, fluid overload is frequently cited as a major contributing factor. The HD Work Group (NKRF) was unable to find a practical clinical measure of estimated dry weight (EDW). The volume status of ESRF patients cannot be directly measured and dry weight is instead defined clinically by exclusion of signs and symptoms indicating overhydration or underhydration.

'Dry body weight' has been surprisingly difficult to define. Zucchelli and colleagues [68] emphasise that the attainment of 'dry body weight', a prerequisite for salt and water control and so for minimising the load imposed on the heart by excess volume, is not achieved as often as it could be. From a conceptual standpoint, dry weight is the postdialysis weight at which the patient is in a state of normohydration or at which the extracellular volume is normal. With few exceptions normalisation of blood volume accompanies this state of hydration and consequently no sign of hyper

or hypovolemia should be found on physical examination or other tests. Dialysis is aimed at restoring such an euvoletic condition by ultrafiltration and the weight at which euvoletism is present is the dry weight. In clinical practice the semantics can get confusing too: dry weight v target weight v post dialysis weight v end-dialysis weight v ideal weight... etc. are attempts in trying to define a state of optimal fluid removal where the goal is subjective, variable and often ill-defined.

Clinicians usually attempt to assess dry weight by determining the lowest possible weight that would not elicit excessive hypotension and muscle cramps during dialysis. It has been stated that a normal predialysis BP without antihypertensive medications can be taken as a gold standard of dry weight. The exclusive use of arterial hypertension can be justified from a prognostic point of view based on studies in the general population and casual office based readings. The latter may not be necessarily true in the dialysis patient.

Using a slightly different approach, dry weight may be defined as the postdialysis weight at which the blood pressure is lowered into the presumed normal range (less than 150 mmHg systolic or less than 90 mmHg diastolic), if possible, without the development of intradialytic hypotension. [69] In addition, the patient should not exhibit signs of pulmonary or peripheral oedema. The emphasis of this approach is on the blood pressure, but the postdialysis weight may be greater than that representing a state of normohydration. The failure to remove sufficient fluid when using this approach could be detrimental because chronic fluid overload adds burden to the heart.

In another approach summarised by Charra et al, [70] postdialysis weight is progressively decreased and fluid removal is progressively increased during long (5 to 6 hours) HD treatments until symptoms of intradialytic hypovolemia and hypotension are consistently observed. Severe muscle cramps frequently occur during this process. After several weeks, however, blood pressure gradually decreases to a level at which all antihypertensive medications can be eliminated, and a dry weight is established. This process has been called “probing for dry weight” and can be an unpleasant experience for both the patient and the clinical staff.

Dry weight has also been defined as the lowest possible weight that would not elicit excessive hypotension and muscle cramps during dialysis. [71] By that definition, of course, virtually everybody who has very large weight gains would be dialysed below their dry weight routinely. Patients who gain above 4 litres will develop hypotension when they are clearly still volume overloaded; i.e. are oedematous and well above their dry weight. This definition can only be used if intradialytic weight gain is not excessive.

Published definitions of dry weight state that the patient should not have orthostatic hypotension at the end of the session. While this may be true for a continuous procedure (like CAPD) it is not true for intermittent HD. However clinical practice of routinely and deliberately provoking hypotension to operationally define a patient's estimated dry weight (EDW) is difficult to justify.

The degree of volume expansion in a dialysis patient is defined historically. It is based on the increase of the patient's weight above the value at discharge from the previous haemodialysis session. However, this assumes that the previous discharge weight was appropriate. For many patients, this assumption is incorrect. For example, the historical EDW may change because of unappreciated alterations in the patient's nutritional status. Alternatively, the historical dry weight may have been erroneous but well tolerated. It is important to realise that dry weight may fluctuate from week to week or month to month. [72] It has to be understood therefore that the hydration status of these patients is in a state of constant flux and never reach a steady state. Assessment of a dry weight is made more challenging by this fact.

BP measurements before dialysis can fluctuate and are affected by factors other than fluid status. Interdialytic weight gains reflect changes in extracellular volume only when effective osmolality does not change. As a decrease in plasma sodium concentration indicate an excess of water in the intracellular compartment, the use of body weight will lead to an overestimation of extracellular volume. In clinical practice sodium concentration seldom show important variations except perhaps in hyperglycemic states. Apart from this situation interdialytic weight gain can be wholly accounted for by salt and water retention.

4b Clinical assessment of fluid status

Relatively few clear-cut guidelines exist for assessing a patient's volume. The amount of fluid that should be removed is often difficult to evaluate and is clinically determined by assigning a dry weight to each patient. Symptoms of dyspnoea, weight gain, lack of appetite (indicating overhydration) and lassitude, dizziness, fatigue, nausea and cramps for underhydration are rather non-specific. Clinical signs are based solely on volume overload or rigorous symptoms of intravascular volume depletion during or post dialysis. The most often used clinical signs to assess fluid status are BP, JVP, oedema, postural hypotension and interdialytic weight gain. Most clinical signs and symptoms are found to be lacking in sensitivity and those few that are relatively sensitive (dyspnoea) tend to be nonspecific. In fact it may be more useful to consider signs and symptoms in terms of their negative and positive predictive values. Signs and symptoms of ECF status can sometimes be discordant.

Raised JVP is a useful sign of elevated right atrial pressure but may be due to hypervolemia or cardiac dysfunction. Oedema clearly typifies the dilemma. Many litres of excess fluid may be present. It may occur early in heart failure, even before rise in JVP and its immediate cause is salt and water retention. However using objective data it has been shown that fluid in patients with intact venous tone, especially young individuals can often be stored in truncal areas that escape clinical detection. Lower extremity venous tone, recumbency, hypoalbuminemia are well known factors that affect fluid redistribution between the trunk and dependent extremities manifesting as presence or absence of oedema. The lack of sensitivity of clinical techniques in being able to detect subtle fluid imbalances has led to the existence of a clinical state of “silent hypervolemia”.

Muscle cramps often accompany IDH and are innocent but extremely unpleasant. They usually involve the lower leg and affect 25% of patients. As with nocturnal cramps the pathophysiology is obscure. Its occurrence is related to ultrafiltration and its rate and is no proof that all excess fluid has been removed. The incidence of cramps does decrease with attainment of dry weight and normal blood pressure [73]

Chest X-ray (CXR) is probably the most sensitive of the available tools in daily clinical practice and can be used to monitor hypervolemia, cardiomegaly, fissural

prominence, pulmonary plethora, Kerley's B lines and often pleural effusions to diagnose fluid overload. However the absence of features of increased blood volume in CXR do not argue against hypervolemia. The positive predictive value is enhanced by presence of an increased cardiothoracic ratio which is the most reliable sign and correlates strongly with UF volume. Despite the number of inaccuracies, it gives valuable information especially with regard to the follow-up of the patient. The accuracy can improve by better standardisation but this has not been attempted so far. Despite these features the diagnosis of congestive heart failure and its distinction from congested state of ESRF may be difficult from a CXR. These changes also take place over a period of time and are of limited value to prescribing fluid removal for each dialysis.

Careful assessment of the underlying pathophysiology of a congested state in ESRF patients by careful clinical examination and radiography alone is a challenge. The methods are rather insensitive in distinguishing normovolemia from silent hypervolemia. Clinical bias favours overload and hypertension because on clinical grounds, physicians will more often overlook saline overload than depletion.

Use of symptom and sign score is subjective, incomplete and non-specific and observer dependent. Symptom Scores can be partly useful but may be fraught with dangers. Scores based on dyspnoea during exercise, recumbency, and sitting and standing, oedema (ankles, tibial) during the previous week, symptomatic dialysis hypotension on the last session has been used in some units in Europe. However their use has only served to highlight the problems of assessing the volume state and limitations of such scoring systems. [74]

Prescription of fluid removal during dialysis requires knowledge of the dry weight and the compensatory mechanisms that allow extraction of the desired amount of fluid during a fixed period of time. Assessment of the latter is lacking in the clinical parameters advocated for assessing fluid status.

4c Inaccurate dry weight estimation - consequences

i) Dry weight too high (The overhydrated patient)

Overestimation of dry weight may have serious consequences. Inadequate fluid removal may lead to fluid overload, hypertension and possible pulmonary oedema.

Fluid overload causes not only hypertension; it is also a potent contributor to left ventricular hypertrophy (LVH), another independent predictor of cardiac death on dialysis. Hypervolemia promotes LVH by increasing left ventricular wall stress. This is brought about not only by increasing cardiac afterload, but also by increasing preload. It is therefore not surprising that regression of LVH could be achieved in haemodialysis patients just by ultrafiltration and reduced salt intake without antihypertensive drugs [75]. The only method systematically proven to be successful in controlling hypertension in dialysis patients is adequate salt and water removal. The benefits of adequate volume control also include improvement in angina, regression in LVH (an independent risk factor) and better efficacy of antihypertensives.

ii) Dry weight, too low

If dry weight has been underestimated -- hypotension and cramps during dialysis, large blood volume changes sustained signs and symptoms of hypovolemia increases morbidity and often culminate in hospital admissions, clotted fistulas and inadequate solute clearance. There are also risks of inducing ischemia to vital organs. Persistently low BP is an independent risk factor for mortality.

Dry weight is clearly a dynamic target that can fluctuate over time. Increased acceptance rates of patients for haemodialysis outpacing the infrastructure (in centre facilities, trained nurses and dialysis doctors) have created busy dialysis units with rapid turnover. It is now accepted that quality of care and appropriate management of a dialysis patient is proportional to the staff and the time available to care for these patients. The increased pressure has a particular impact on ultrafiltration and fluid balance. The empiricism and lack of close monitoring partly accounts for some of the difficulties in maintaining dry weight and achieving satisfactory BP control. Scribner commented "Dry weight and the normal blood pressure that goes with it is a constantly changing value that must be redetermined with each dialysis." This emphasises a hugely important aspect of the problem.

Chapter 5

Body Fluid compartments and their relationship

5a Compartmental distribution of body water

One century after Claude Bernard described milieu interior, the anatomy of body fluid compartments were described. [76,77] Approximately 60% of the body is composed of water; two thirds of total body water is intracellular, and one third is extra cellular. [78] Extracellular fluid can be further divided into the plasma compartment, the interstitial compartment, and the transcellular compartment. (Fig 12) The transcellular compartment is normally a fraction of the extracellular fluid and is often neglected. However it may be an important in ESRF patients since it includes the peritoneal cavity. Approximately 1/5 of the extracellular fluid is located within plasma and four fifths located extravascularly. Blood volume, comprises of erythrocytes, other formed components and plasma. Albumin is the main marker of plasma. The proportion of blood taken up by erythrocytes is called haematocrit.

A healthy nonobese man of 70kg has approximately an intracellular volume of 28L(ICF), an extracellular volume of 17L(ECF), a plasma volume of 3L and an erythrocyte volume of 2L, and as a consequence a haematocrit of 0.4(40%). If we subtract the plasma volume from ECF, the resulting volume is interstitial volume, which in this case 14L.

The volume of total body water can be determined with an accuracy of about 3% (± 1.5 L) by determining the dilution of a marker substance that distributes throughout this space, such as isotopic water or antipyrine. [78] As measured by such techniques, the fraction of body weight that consists of water varies considerably among individuals and depends on several factors: age, gender, body fat content, and the presence of altered physiological or pathophysiological states. The determination of extracellular fluid volume by dilution techniques is less rigorous because an adequate marker substance that is exclusively confined with compartment does not

exist. Dilution techniques have not routinely been evaluated in ESRF patients. It is often assumed that the distribution of ESRF patients is similar to normal individuals but this contention has not been adequately examined.

The cell membranes separate ICF from ECF and are freely permeable to water. The normal inter-relationship of these compartments is maintained by selective permeability to osmotic solutes. Despite the different composition of solutes, the osmolality of the ICF and ECF compartment is always the same. Any change in extracellular osmolality will immediately be followed by similar change in intracellular osmolality. Adding pure water to ECF will cause majority of it to move to ICF because it is larger. Conversely water withdrawal will mainly cause decrease in ICF.

Aquaporins (AQP) are integral membrane proteins that serve as channels in the transfer of water, and in some cases, small solutes across the membrane. They are conserved in bacteria, plants, and animals. Structural analyses of the molecules have revealed the presence of a pore in the center of each aquaporin molecule. The prime function of aquaporins (AQPs) is generally believed to be that of increasing water flow rates across membranes by raising their osmotic or hydraulic permeability. In mammalian cells, more than 10 isoforms (AQP0-AQP10) have been identified so far. They are differentially expressed in many types of cells and tissues in the body. Aquaporin 1 is present in capillaries and venules and appears to be important in peritoneal dialysis, where it appears to represent the "ultrasmall pores" of the three-pore model. Furthermore, during glucose-induced osmosis during PD, nearly 40% of the total osmotic water flow occurs through molecular water channels, termed "aquaporin-1." Decreased expression or function of AQP1 may be responsible for some cases of ultrafiltration failure, but further evidence will be required to establish whether this is the case.

Although a large number of particles constitute plasma, the only quantitatively important solute are sodium and its accompanying ions (Cl^- and HCO_3^-). ECF is determined by the total body sodium [$\text{ECF (l)} = \text{total body sodium} \div 140$]. Hence when ECF is expanded, a large excess of Na is also present. Determination of sodium concentration does not give us an idea on Na content of the body. Blood sodium concentration is a reliable estimate of cellular hydration.

Osmolarity indicates the number of osmotic particles in a litre of the solution, while osmolality is expressed per kg of water. There is approximately a 7% difference

between these measures in blood and plasma (lipoproteins). Men and women differ significantly with respect to their water/weight ratio; women have a lower body water content when expressed as a fraction of body weight. Body fat has a low-water content and makes up a larger fraction of body weight on average in women, which accounts for the gender difference. Use of V as a denominator essentially eliminates body fat content as a deciding factor for dosing dialysis. In other words, body fat is acknowledged not to be a determinant of need for dialysis, and in accordance with the concentration toxicity theorem, fat would not be a source of uremic toxins. Although fat is a major component of all cell membranes and is especially prevalent in neural membranes, the majority of fat in the body is found in adipose tissue that is distributed primarily in the skin in which it is found in more abundance in women compared with men. Adipose tissue has long been considered an inert tissue serving as a repository for energy storage and heat insulation. More recent studies suggest that adipose tissue is more active metabolically than was previously appreciated, so eliminating fat as a source of uremic toxins could be premature. [79] On the other side of the coin, body edema fluid, which represents an excessive expansion of the extracellular volume and is probably more inert than adipose tissue, is included in the denominator. Body water content is increased in edematous individuals, but it is unlikely that this addition to total body water increases the need for dialysis. So it is reasonable to question the wisdom of using urea distribution volume as a universal denominator for dosing dialysis.

The interstitial space

Approximately 10 litres is contained in the interstitial space. The volume of this compartment is generally expanded in order to accommodate excess fluid accumulation. The fluid is bound in a proteoglycan matrix, which prevents fluid from flowing easily through tissues. The proteoglycan elements behave rather like a sponge, which maintains the compartment at a slightly negative pressure. In the nonoedematous state this heterogeneous system has a gel fluid property. (Fig 13) The interstitial space thus serves as a reservoir of extracellular fluid that can be mobilised in the event of transient loss from the circulation. The expanded space in overloaded dialysis patients give rise to interstitial pressure causing oedema. Under these conditions, fluid moves easily through the very compliant subcutaneous tissue

underneath the skin. Large volumes of fluid may be stored without increasing the interstitial pressure significantly. [80,81,82]

5b Control of body fluid volumes

Regulating mechanisms in normal man

i) Antidiuresis, thirst and salt

An important factor to control homeostasis preserved in dialysis subjects is the appetite for salt. Stricker [83] pointed out that osmotic dilution stimulates salt appetite in rats and this is probably as important as a mechanism for control of body fluid volumes. Decrease in plasma volume and increase in osmolality, the same factors that affect antidiuretic action also stimulate thirst.

ii) Natriuresis

Extracellular space expansion particularly by saline infusion in normal subjects is associated with marked natriuresis. This effect occurs without much plasma volume expansion but is highly correlated with an increase in interstitial fluid volume. The effect is promoted more by changes in the sodium load than by changes in the volume load per se. Whether or not a natriuretic factor exists is still not fully explored. ESRF patients are most unfortunately deprived of such protective natriuresis.

iii) ECF regulation

Volume regulation is dependent on sodium excretion by the kidney, which is influenced by an integrated regulatory system in which blood pressure and renin-angiotensin-aldosterone play the most important roles. [84]

In normal man kidneys are constantly engaged in excreting the excess ingested with the food, over and above the minute losses by sweat and stools. The anephric patient is thus constantly threatened by extracellular overhydration.

The ability of the kidney to excrete salt is limited. Overhydration tends to occur when the amount that needs to be excreted is more than 5% of the filtered load. When kidney function drops to below 20% of normal, 5% of the filtered load represents less than salt content of normal diet. The regulation of ECF is not fully understood. Changes in blood volume or ECF by an afferent pathway mediate pressure natriuresis (efferent pathway). This mechanism is closely regulated by RAS. This is activated by hypovolemia and inhibited by hypervolemia. Despite the fact that abnormalities of the RAS play an important role in hypertension, this system seems to be primarily related to both salt and volume homeostasis. This is important, since as discussed in next chapter, in dialysis patients normal BP can be achieved in a large majority by manipulating their volume state. Normotension can be associated with a broad range of renin-angiotensin levels. However hypervolemia leads to hypertension even in the absence of renin.

iv) Osmoregulation

Osmolality is regulated by the kidney, which can produce urine containing more or less water than body fluids. However normal osmolality can be maintained in the absence of kidney function.

The extracellular osmolality (sodium concentration) indirectly controls the ICF. This is maintained by two mechanisms, the regulation of thirst and by the ability of the kidney to produce hyper or hypotonic urine mediated by ADH. [84] This is an amazingly precise and efficient control system that is rapid and sensitive.

v) Regulation of interstitial fluid volume

The interstitial fluid volume can be regulated precisely. The interstitial fluid exists in two phases: as free fluid and as fluid imbibed in a gel like ground substance. Under normal conditions essentially all the fluid is in the latter form. When free fluid increases even slightly, lymphatic flow also increases tremendously thereby acting normally as a negative feedback mechanism to prevent any significant increase in free fluid in the tissue spaces. Lymphatic flow removes protein and water from the tissue spaces decreasing tissue colloid osmotic pressure and increasing capillary reabsorption of fluid. [83] The interstitial fluid gel on the other hand pulls fluid from

the free fluid phase. Its imbibition forces determine the amount of fluid held in the gel and this fluid quantity is stable at least for short-term haemodynamic function. (Fig 13) The lymphatic system and capillary reabsorption normally maintain the interstitial spaces relatively dry of free interstitial fluid. The normal interstitial fluid volume is almost entirely that volume imbibed in the tissue gel. When the normal drying mechanism for the interstitial spaces fail to maintain the dry state, then large quantities of free fluid begin to collect and oedema ensues. [82]

Chapter 6

The intravascular compartment and its regulation in haemodialysis

Haemodialysis treatment removes surplus body water from overhydrated tissues in addition to removing toxic substances and metabolites. The total amount of fluid removed during a single dialysis session may vary from 1.5 – 4 litres. As plasma volume is only 4.5 – 5 % of body weight at a haematocrit of 30%, the total ultrafiltered water removed during a single dialysis session may in some cases exceed the total circulating plasma volume. To preserve haemodynamic stability, physiological responses intervene such as a fall in venous capacitance and an increase in sympathetic activity to maintain blood pressure. Similarly the fluid retained in the interdialytic period is redistributed in the extracellular compartment through passive and active blood volume regulatory mechanisms to maintain haemodynamic stability.

6a Effects of fluid retention on the blood volume compartment and cardiovascular system

An increase in the ECF that results from salt and water retention will lead to a cascade of events that affect the blood volume compartment and its dynamics. Because blood volume and ECF are so closely linked, the terms fluid retention and overfilling are often used interchangeably. In general the terms fluid retention and overhydration are used to describe an increase in the ECF while hypervolemia describes an increase in the blood volume.

As plasma is a part of the extracellular volume, plasma volume and therefore blood volume change concomitantly with the ECF. Because the Starling forces, (Fig 14) which govern the partition between BV and the interstitial compartment, may change, this relationship is not the same in all circumstances. For instance in

conditions with hypoalbuminemia BV increases are less despite large increase in the interstitial volume.

The ECF – BP relationship is not linear. [78] BV increases only slowly when ECF expansion exceeds 5L, which is the amount when oedema appears. It is evident that the volume of blood within the vascular system cannot increase indefinitely although there is no apparent limit to expansion of ECF.

The increased volume will be distributed within the cardiovascular system according to the relative compliance of its different compartments. In addition the elasticity of the vascular compartment, which has muscular walls, may actively change compliance. As the venous system has the largest compliance it will be affected first, with a small but a significant increase in pressure. There will also be an increase in the diastolic dimensions. The diameter of the heart on CXR decreases 0.5-1 cm after UF 2-4 L during dialysis. According to Starling's law, hypervolemia must be accompanied by increase in cardiac output and a hyperdynamic state. Fluid retention leads to hypervolemia, which leads to hypertension. The rise in BP as ECF increases parallels the ECF – BV relationship. The relationship is non-linear suggesting that the main determinant of blood pressure is blood volume. The hypercirculatory state causes hyperperfusion of tissues; the blood delivers more oxygen which downregulates blood flow by vasoconstriction. The autoregulation leads to increase in TPR and hypertension.

Guyton's Auto regulation Concept - Relationship of BP to BV

The fluid status-BP control relationship is usually explained by Guyton's concept of autoregulation of systemic blood flow [85]. The basic pathophysiology of this concept is that fluid homeostasis is maintained at the expense of BP elevation. According to this theory, blood flow to peripheral tissues is regulated by changes in local vascular resistance. Any increase in cardiac output induced by expansion of extracellular or blood volume (or both) augments peripheral blood flow, which in turn elicits an increase in peripheral vascular resistance to restore local blood flow toward baseline. The normal operating point for a 70kg man with the ideal fluid status is 5 litres of blood volume and 15l of extracellular fluid. Under these

conditions, the patients BP is typically within a normal range. If a blood volume becomes depleted below the normal value, it is clear that this will be insufficient to maintain a BP, leading to hypotension. In the opposite direction from the normal operating point, then initially, blood volume rises linearly with ECF eventually leading to hypertension in order to keep BP to certain limits.

At "high-normal ECF" a very small saline load suffices to trigger a wide BP increase. On the other hand, at this higher basal ECF, intradialytic hypotension is less likely. The practical evaluation of the ideal ECF at a given time rests on attaining the ideal postdialysis weight.

However, studies of the relationship between volume status and BP are not conclusive. Whereas de Planque et al. [86] found a good correlation between BP and extracellular volume in hypertensive patients who had advanced renal failure and were not yet treated with dialysis, Dathan et al. [87] could not confirm these findings in a study of 10 dialysis patients who underwent salt and water loading only one patient followed the classic pattern of Guyton's autoregulation hypothesis. In two patients, BP did not change after increasing dry weight. Schultze et al. [88] studied haemodialysis patients who were normotensive and those who were hypertensive in spite of efforts to reduce dry weight. The hypertensive patients were found to be no more volume-expanded than the normotensives.

Autoregulation and hypertension

Dialysis patients are constantly threatened by fluid overload and it would therefore be remarkable if most of them did not suffer from some hypervolemia. Increased BV leads to a slight increase in right atrial pressure. A normal heart reacts to this increased 'pre-load' according to the Frank-Starling law by increasing cardiac output (CO). This hypercirculation induces vasoconstriction in the tissues (so called autoregulation), that increases BP further [89].

Although a hypercirculatory state is present in many dialysis patients it is not easy to prove that this is caused by fluid overload because of confounding factors like *anaemia* and A-V fistulas. It is even more problematic to prove that this also leads to hypertension along the sequence shown above. While one author described these events in a dialysis patient, others did not detect an increase in cardiac output when BP rose after overhydration, while in some patients BP did not increase at all [90].

Nevertheless, whenever hypertension develops after volume expansion, it is always characterised by an increase in total peripheral resistance. This, in turn, leads to a search for vasoconstrictive substances and drugs that cause vasodilatation and the use of drugs that cause vasodilatation.

Reflex control of the circulation

A detailed discussion of blood pressure control is well beyond the scope of this thesis, however there are a few aspects worth highlighting, since blood volume and blood pressure are intrinsically linked. While excessive blood volume can affect blood pressure, there are many mechanisms allowing blood pressure to be controlled independently of blood volume i.e. vagal afferent stimulation, chemoreceptors and baroreceptor system in man. Kumada and Sagawa demonstrated that blood volume changes of 10 –20 % cause a 21-31 % change in impulse traffic in the rabbit aortic nerves with only a 6 % increase in arterial pressure. Therefore they suggest that arterial baroreceptors act as volume receptors in the same way as atrial receptors. [91]

In view of the great emphasis placed on pulse pressure as a stimulus to baroreceptors in recent years Kumada et al demonstrated that the pulsatile component of the carotid sinus reflex does not improve the reflex response of an animal to haemorrhage. This experiment was performed by preventing pulses from reaching the carotid sinus area. [92] Vascular stretch reflexes exert their effects only during the first few hours to the first few days after pressure changes. Long-term regulation depends on chemoreceptor reflexes or intrinsic control of the circulation itself.

Autonomic control

The failure of normal compensatory mechanisms to sustain adequate venous return results in a drastic reduction in right ventricular volume. In susceptible individuals the acute reduction in ventricular volume is accompanied by a paradoxical increase in afferent inhibitory output of the cardiac mechanoreceptors, which abruptly decreases the sympathetic output. A second potential pathway involves the activation of the parasympathetic system (Bezold-Jarisch reflex). [93] Both pathways result in hypotension with relative bradycardia and are akin to vasovagal syncope. Non-

diabetic patients have normal baroreflex function and the acute hypotensive episodes were linked to paradoxical withdrawal of sympathetic activation similar to vasodepressor syncope.

Compliance

The best way of describing the factors determining the distribution of volume excess is the compliance of the compartments, a kind of elasticity. A high compliance indicates a high elasticity, while low compliance stiffness. Mathematically this is defined as change in hydrostatic pressure, which occurs at a given change in volume (P/V).

Compliance may change in pathological conditions. For instance, the interstitial space normally has a rather low compliance with a negative pressure and exists in a gel like state, which is not freely movable. With accumulation of fluid when oedema appears the gel structure is broken and many litres of fluid can accumulate without a measurable change in interstitial pressure. The equilibrium between the blood compartment and the interstitial volume depends on the difference in their pressures the former or intracapillary pressure principally determined by precapillary vascular resistance and the venous pressure. The dynamic nature of the compliance explains why the Guyton curve is non-linear. The haematocrit, compliance and the function of the heart, which vary from patient to patient may have important influences on the ECF/BP relationship in dialysis patients and has not been studied.

6b THE MICROCIRCULATION

The total blood volume can be physiologically described as comprising of two haemodynamic compartments. The large arteries and veins are termed the macrocirculation. The microcirculation on the other hand is defined as one including all microvessels with a diameter smaller than 250 μm . The microcirculation is distensible, harbours 40 – 50% of the total blood volume and more than 90% of the total peripheral flow resistance. The microcirculation is the principal site of transcapillary exchanges involving the delivery of oxygen, the removal of carbon dioxide and metabolic wastes and the transport of fluid flux and solutes. Other than mass transport, the microcirculation and endothelial cells have a role in regulation, signal transduction, proliferation and repair. The microcirculation plays a more important role as a reservoir of blood volume than the venous system. Microvascular pooling rather than hypovolemia is a likely factor in many instances of hypotension in HD and in endotoxin shock. Understanding the role of the microcirculation could lead to more effective diagnosis of cardiovascular deficiency and therapy for hypotension or low cardiac output with intervention through the microcirculation. [94]

Smooth muscles surround the arterial end of the capillary, called the arteriole, which behaves like a valve allowing the flow of blood through the capillary to be regulated. A venule at the venous end of the capillary has a similar function. By adjustment of the arterioles and venules both the resistance of the capillary to the flow of blood and the pressure inside the capillary may be controlled. Consequently the combined microcirculation may perform a vital role in the control of blood volume and blood pressure. Blood from the microcirculation passes into the veins and the great veins, which provide considerable degree of vascular compliance. This elastic capacity of the vascular space allows it to contract during reduced blood volume to maintain pressure.

With the development of intravital microscopy, Chambers and Zweifach [95] mapped out the intravascular network and marked the distribution of vascular smooth muscle in arterial walls or around some capillary entrances. They observed that the arterioles and capillary sphincters could constrict to alter the degree of perfusion and affect the flow heterogeneity of the network, leading to the suggestion that the

microcirculation has the capability to regulate blood flow locally. Integration of this local regulation from individual microvessels to the whole organ allows autoregulation of the total blood flow. Neural and humoral factors along with autoregulation control the performance of the circulation.

The mapping of the microvascular networks in thin tissues such as the mesentery, cremaster, and gracilis muscle and the subsequent modelling of the microvascular flows indicate that each tissue may have a different network organisation, capillary density and pressure distribution. The microvascular function of the heart, lung and liver has been studied with their surface vasculature, fixed tissue samples or indicator dilution methods. Morphometric measurements from three organs have established that the microcirculation could contain 40 –50% of blood volume. Multifocal X-ray imaging and a new generation of contrast agents are now available for assessing the heterogeneous microvascular or regional flow of solid organs in vivo or in vitro. These could lead to precise characterisation of microvascular morphometry, exchange and regulation.

The endothelial cells can release endothelin and nitric oxide to regulate the blood vessels downstream. The cells are in communication with each other and play a pivotal role in signal transduction. This role may be influenced by mechanical stresses imposed by blood flow. Endothelial cells may play a role in the growth of microvessels as collateral channels improving blood flow. Since most endothelial cells reside in the microcirculation, this provides an effective site for drug and gene targeting with therapeutic benefits.

The morphometry of the pulmonary vascular networks was mapped out by Horsfield and Gordon [96]. The arterial macrocirculation has two vessel groups: arteries ($d > 2\text{mm}$) and the small arteries ($2\text{mm} > d > 250\mu\text{m}$). The microcirculation consists of three groups: the arterioles ($250\mu\text{m} > d > 8\mu\text{m}$), the capillaries ($d < 8\mu\text{m}$), and the venules ($250\mu\text{m} > d > 8\mu\text{m}$). (Fig 15) Finally the two groups in the venous macrocirculation are the small veins ($2\text{mm} > d > 250\mu\text{m}$) and the veins ($d > 2\text{mm}$). The capillaries of the heart and lung contain the majority of the microvascular blood volume and their arterial macrocirculation is volumetrically comparable with their venous macrocirculation. The mesentery has most of its microvascular blood volume in the venules and most of its macrovascular volume in the veins and small veins. The distensibility of the microcirculation is comparable to the macrocirculation.

6c Blood volume regulation during Ultrafiltration

During HD (combined diffusion and ultrafiltration) the plasma water removal induces a progressive reduction in blood volume. Hemoconcentration is followed by a fluid shift from the interstitial and cellular spaces toward the vascular compartment. Hypovolemia triggers the sympathetic system, which induces vasoconstriction and an increase in myocardial contractility and heart rate. The maintenance of blood pressure is related to two fundamental mechanisms, blood volume preservation and cardiovascular compensation. Arterial hypotension occurs when central hypovolemia causes an underfilling of the cardiac chambers, thereby compromising the circulatory load, while either the vascular arteriolar or venous tone decreases or is inadequate in relation to the stroke volume reduction. In a stable HD patient, the most critical period of instability is in the latter half of the session. The mechanism that explains this is failure of microvascular resistance vessels to constrict during treatment in response to a declining vascular volume.

i) Blood volume and intradialytic hypotension

The blood volume in a typical dialysis patient varies from 4.5 to 6l, with a corresponding plasma volume of 3 – 4 l. In patients undergoing thrice weekly dialysis schedules and gaining 1.5kg/d, the therapeutic requirement is to remove 3 l of fluid per dialysis treatment or nearly the entire plasma volume. It is a tribute to the compensatory mechanisms present in the body that patients routinely not only survive such a marked insult to their circulatory system, but also do so with little decrease in BP. The compensatory mechanisms are cardiac, plasma refilling, passive venoconstriction and active increases in arterial tone.

Further more when fluid is ultrafiltered from the vascular compartment during HD, intercompartmental shifting of intracellular and extracellular fluids occurs. In disequilibrium syndrome, hypotension, and other symptoms, and these complications reflect the poor physiological compatibility of rapid fluid removal during HD.

The changes in cardiac rate in response to hypovolemia are often impaired in dialysis patients. [97] Whereas cardiac rate changes may be important in increasing cardiac output from baseline (e.g., during physical activity), they appear to be less important in maintaining cardiac output under conditions of decreased filling. For example, studies in dogs receiving propranolol during haemorrhage indicate that this cardiac rate change is of minimal importance in maintaining cardiac output during hypovolemia. Another cardiac compensatory mechanism is increased contractility. [97] Whether this is of clinical importance during hypovolemia is open to question. A number of electrolyte changes that occur during dialysis (decrease in serum potassium, increase in serum bicarbonate and change in serum ionised calcium) have been reported to affect cardiac contractility. [93, 98] Manipulating these ion changes (e.g., increasing dialysis solution calcium levels) has been reported to increase intradialytic blood pressure [98], but it is unclear if the BP effect was due to cardiac causes or to calcium effects on the peripheral vasculature.

ii) Concept of Plasma refill

The primacy of plasma refilling is indicated by the fact that, if one does not remove fluid during dialysis, intradialytic hypotension is rare. [99] This emphasises that the fact that the ultimate cause of hypotension is reduced cardiac filling. The initial compensatory mechanism is refilling of the plasma water space from surrounding tissue spaces. As a result of refilling, even though the entire plasma volume is removed during dialysis, the blood volume typically decreases only by 5% to 20%. The source of refilling fluid is primarily the extracellular space, although this depends on the dialysate sodium level. Evidence suggests that during HD, patient differences exist in vascular refill relative to the rate of ultrafiltration [66]. The plasma refill rates have been indirectly estimated as high as 1500ml/hr in moderate hypervolemia. [100] Using a higher dialysis solution sodium concentration will result in mobilization of intracellular water for this purpose and better maintenance of the plasma volume during dialysis. From practical experience, one often finds that plasma refilling is also enhanced with a high plasma albumin level and in conditions of fluid overload. Mobilization of ascitic fluid by dialysis is difficult, because the

rate of equilibration between intraperitoneal fluid and the plasma space tends to be quite slow.

iii) Transvascular water and solute exchanges

The hydrostatic forces within the capillary beds may also affect refilling. (Fig 15) Any vasodilator (e.g. acetate) that serves to dilate arterioles and cause transmission of increased hydrostatic pressure to the capillary bed, will also act to inhibit refilling.

Two approaches have been used in descriptions of net transfer of solutes and water. One approach has been the classical approach of Starling forces, vascular and interstitial hydrostatic and oncotic forces and on the concept of filtration in bulk without separation of solvent water from small solutes. The latter determined by factors, in addition to oncotic and hydrostatic forces considered in the original Starling hypothesis, such as hydraulic conductivity, capillary surface area, reflection coefficient for plasma proteins and the relationship of protein flux to the volume flow across the capillary. In a second physicochemical approach based on nonequilibrium thermodynamics and on multiple indicator-dilution experiments, diffusion is considered the dominant mechanism in transvascular exchanges of water with net transfers related primarily to the permeability and net passage of small solutes such as sodium and chloride ions (osmotic buffering). Reconciliation of two approaches is in the hypothesis that small solute controls are dominant over a period of seconds while oncotic factors are operative over minutes. These estimates are based on linear models of extravascular distributions and for permeability calculations. Aquaporins may be important but their role has not been completely defined. [101]

iv) Venous capacity: interaction with arterial tone

Detailed haemodynamic analyses of intradialytic hypotension provide two important facts. (1) There is no sudden absolute decrease in plasma volume just before a hypotensive episode. (2) Intradialytic hypotension seems to be due to a decrease in cardiac output engendered by reduced cardiac filling. [102]

Most of the 3L or so of plasma volume resides in the veins, and several organ perfusion systems, notably skin and splanchnic, contain veins that can markedly alter their capacity. A slight loss in venous tone in either of these systems can result in a marked ebbing of flow return to the heart with loss of cardiac filling and a resultant decrease in cardiac output. Venous tone in the circulatory system is affected by vasoactive hormones and the sympathetic nervous system (SNS), but also responds to upstream filling pressure; i.e. enhanced transmission of upstream arterial pressure to the veins via the capillaries will cause the veins to distend, increasing their volume. This is the basis of the so-called De-Jager Krogh phenomenon [93] and explains how pathophysiologic conditions or drugs that cause arterial dilatation may also result in venodilatation and alterations in venous capacitance.

Thus, some episodes of dialysis hypotension, especially the sudden hypotension that occurs without a marked antecedent increase in hematocrit, might be due to sudden relaxation of splanchnic veins causing an abrupt reduction in cardiac filling. So, what are the practical aspects of maintaining venous tone? Arteriolar constriction must be maintained. The use of acetate was abandoned because of an increased incidence of dialysis hypotension and other side effects. Interestingly, acetate and food ingestion has the specific effect of increasing splanchnic blood flow and dilating splanchnic blood vessels.

There is an interesting hypothesis which suggests splanchnic blood pooling and the resultant dialysis hypotension is related to adenosine. When ATP (adenosine triphosphate) is used by the cell, adenosine diphosphate and monophosphate (AMP) are generated, and then AMP is metabolized to adenosine. Adenosine is then further metabolised to inosine and hypoxanthine and, ultimately, to uric acid. Because adenosine is difficult to measure, adenosine turnover or generation is often measured by serum levels of its metabolites, inosine and hypoxanthine. Woolliscroft and Fox [102] showed that patients who were hypotensive for a variety of reasons had increased levels of inosine and hypoxanthine in the blood, presumably due to ischemia-induced consumption of adenosine triphosphate (ATP) with increased generation of AMP and adenosine. It was also noticed that use of acetate containing dialysis solution increased adenosine products in the circulation. The explanation for adenosine causing hypotension is thought to be mainly the inhibitory effects of

adenosine on norepinephrine release. This may be a physiologic mechanism, in that a tissue that is not getting enough oxygenation releases adenosine, causing regional vasodilatation and increased local flow. The concept has been put forward that a "vicious cycle" may occur in some patients with sudden hypotension: hypotension induces ischemia, which, in turn, results in increased local adenosine release, which then causes inhibition of norepinephrine release and vasodilatation, which results in deepening hypotension. Dialysis patients who had sudden decreases in BP intradialysis (but not in patients exhibiting gradual BP reduction) hypotensive episodes are preceded by the appearance of increased levels of inosine and hypoxanthine in the blood. Shinzato et al [103] then went on to pretreat these patients with caffeine (an adenosine blocker) in a crossover study and found that they experienced fewer decreases in blood pressure during dialysis.

The degree of peripheral arteriolar constriction also affects the blood pressure by a second mechanism that is independent of venous capacity and cardiac filling. This, of course, is a direct hemodynamic effect: at any level of cardiac output, the level of arteriolar tone will determine the level of central blood pressure.

In addition to a variety of vasoactive hormones, the principal control mechanism of arteriolar tone for resistance vessels has to do with the level of SNS activity. The level of peripheral sympathetic nerve activity can be measured by means of a small electrode inserted close to the peroneal nerve. Such measurements have determined that ESRD patients have an increased basal level of peripheral sympathetic nerve activity. [45] Because this is not found in ESRD patients who have undergone bilateral nephrectomy, it has been hypothesized that this excess sympathetic activity may be generated in some way via afferent renal nerves originating in the diseased kidneys. During dialysis, as fluid is removed, increased peroneal sympathetic nerve activity has been measured. This is thought to be an appropriate response. However, in hypotension-prone patients, Converse et al [57] found a paradoxical decrease in SNS activity at the time of hypotension. The measured SNS activity predialysis, 5 minutes before a hypotensive episode, and during the hypotensive episode. When hypotension supervenes in these patients, there is a paradoxical decrease in SNS activity. In another study, SNS activity was imputed from spectral analysis of heart

rate variability based on the low frequency and high frequency heart rate changes. [104]

6d Application of blood volume monitoring in HD

The overwhelming need for objective quantification of fluid status has not been realised despite many other advances in dialysis technology. It is clear a common final pathway of blood volume reduction is central to hypotension during ultrafiltration whatever the aetiology. This has been the primary motivation for the development of blood volume monitors. The first continuous blood volume monitor was developed over 15 yrs ago by Stiller et al. [105] Since then a variety of techniques have been devised employing principle of optics, ultrasound, viscometry and conductivity described in further details in the subsequent Methods section. Blood volume monitors have now become an integral and standard feature of most modern dialysis machines. The availability of this technology has been the source of renewed interest in methods to quantify fluid status and achieve more physiologically appropriate fluid removal during dialysis. Several types of monitors are available based upon different principles and offering various degrees of precision and immunity to artefacts. These have been detailed in the subsequent Methods section. For the past few years relative blood volume measurements have been possible with devices based on the optical reflection and densitometry methods. Since then various authors have tried to determine individually determined decrease of relative blood volume at which the patient will develop hypotension. It has been proposed that the degree of blood volume reduction as a consequence of ultrafiltration may reflect the fluid status of a patient. Blood volume changes have been associated with BP changes, [68] hypotensive events and hydration status [106]. Most studies have tried to link the fall in blood volume to a fall in blood pressure, proposing that blood volume changes could be a predictor of hypotension. Generally the results of such studies have been inconclusive and failed to provide clear-cut guidelines. [107,108] Although maintaining blood volume above a critical limit does not pose any major technical difficulties using advanced feedback control systems, a number of shortcomings have limited its acceptability and widespread usage [109] Wojke et al

could show that in 7.2% of prone hypotensive relative blood volume showed a high interindividual variation, ranging from 96 to less than 86%. [110]

Continuous blood volume measurements and determination of the relative blood volume variations and limits are of importance in relation to regulation of ultrafiltration volume and its prescription. The possibility of feedback control of blood volume during HD has been proposed as this may enable fluid to be removed during each session on an individualised basis. Current blood volume control strategies are relatively crude. The existence of a critical blood volume remains to be convincingly proven in dialysis patients. Quantitative blood volume parameters to assess hydration in real time during dialysis instantaneously are lacking despite the available technology. The limitations of current strategies are due to various factors.

The relative blood volume characteristics during UF have not been defined and its interpretations remain unclear. Hydration status is not the sole factor affecting stability. Temperature, electrolyte balance, and other cardiovascular compensatory systems all play a part in maintaining intradialytic stability. As a result the blood volume changes have to be interpreted during a single treatment by the given state of these variables. Blood volume control systems that have been developed do not involve methods which characterize individual patient responses to fluid removal but rely on predefined trajectories. Finally the true relationships between a relative and the absolute changes in blood volume during ultrafiltration have not been studied.

Achieving dry weight and avoiding hypotension has to also take into account other fluid compartments where excess fluid resides and compensatory mechanisms induced by fluid removal in these patients. One important element lacking in current blood volume strategies is a suitable physiologic model of the HD patient during ultrafiltration and the forces that govern alteration in haemodynamics. The use of dynamic tests based on ultrafiltration pulses and intradialytic monitoring may allow us to understand the ultrafiltration pathophysiology. Insight into these mechanisms may then provide the opportunity of individualising the ultrafiltration prescription.

Table 1 Common causes of ESRF

Causes	Eng & Wales 1995	USA 1991 -1995	Australia 1996	Japan 1994
Glomerulonephritis	12.4	11	34	39.8
Diabetes	13.8	37.4	18	31.2
Cystic disease	5.9	3.5	7	2.6
Hypertension	7.8	28.7	12	6.2
Pyelonephritis	9.1	4.5	4	
Analgesic			7	
Missing	17	4.4	7	
Miscellaneous	18.1	6.2	11	

Table 2 Excellent BP control reported in certain dialysis centres

Author	Location	HD modality	Year	Normotensive patients (%)	% on AH drugs
Thompson [40]	USA	LHD	1970	90	-
Charra [31]	Tassin	LHD	1997	97	3
Goldsmith [281]	Manchester	LHD	1998	98	2
McGregor [42]	Christchurch	HHD	1999	94	8
DorhoutMees[43]	Turkey	CHD	1996	90	1
Ozkahya [75]	Turkey	CHD	1998	86	-
Kooman [175]	Maastricht	CHD	1999	75	-

AH antihypertensive drugs ; LHD long hours HD ; CHD conventional HD ; HHD Home HD

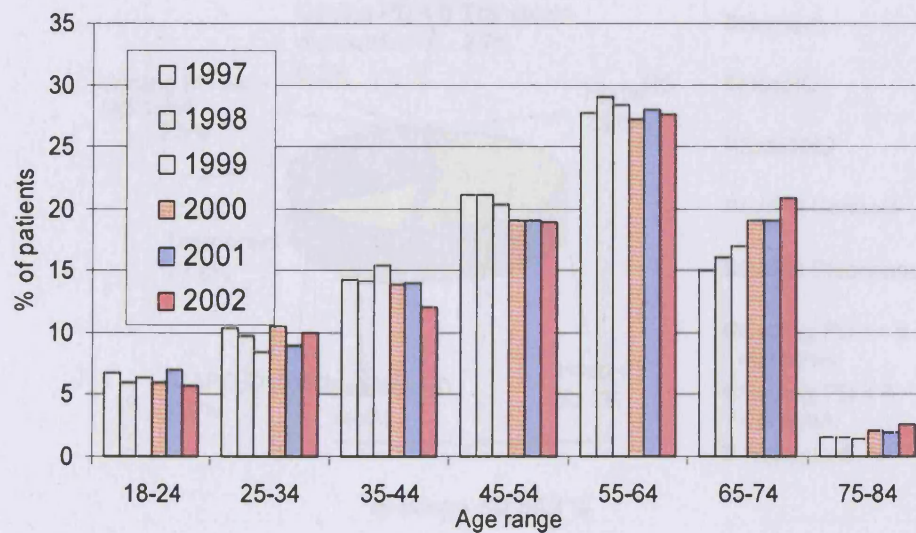
Table 3 Causes of hypertension in patients undergoing Haemodialysis [39]

Fluid retention
Pre-existing essential hypertension
Increased sympathetic activity
Deranged renin-angiotensin system
Hypercalcemia
Increased plasma-endothelin 1
Disordered Nitric oxide metabolism

Table 4 Intradialytic complications by patient age ; Percentage of dialysis treatments with specific symptoms[55]

	Age groups (yrs)		
	< 30	30 - 50	50 - 70
Number of treatments	1314	5355	11085
Percentage of patients with :			
Hypotension	18.1	19.7	25.2
Nausea	8	6.8	8.1
Vomiting	3.4	2.3	3.7
Cramps	11.4	13.3	10.2
Chest pain	0.9	1.2	1.5
Fever	0.6	0.2	0.2

Figure 1 Percentage of new patients by age group, E&W1997- 2002 (Renal Registry UK)



Changes in PD and HD Comorbidity
CMSS 1986 and DMSS 1996 Incident Cohorts
USRDS ADRS

	<i>PD</i> <i>1986</i>	<i>PD</i> <i>1996</i>	<i>HD</i> <i>1986</i>	<i>HD</i> <i>1996</i>
% DM	45	48	41	50
% CHD	38	36	41	42
% CHF	36	31	41	42
% PVD	22	20	19	23
% CA	6	8	9	11

Figure 2 Increasing Comorbidity in the ESRD population accepted for Dialysis USRDS [2]

Figure 3 Percentage of dialysis patients on hospital based haemodialysis (UK Renal Registry RRT Modalities, day 90, 2002 cohort)

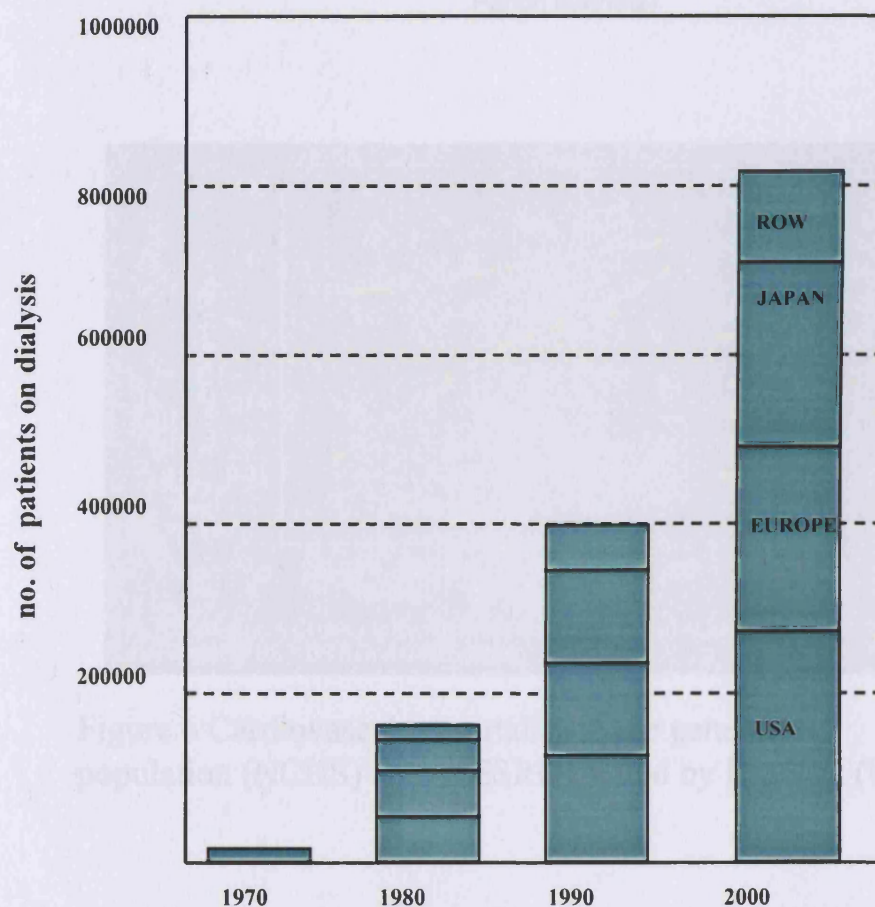
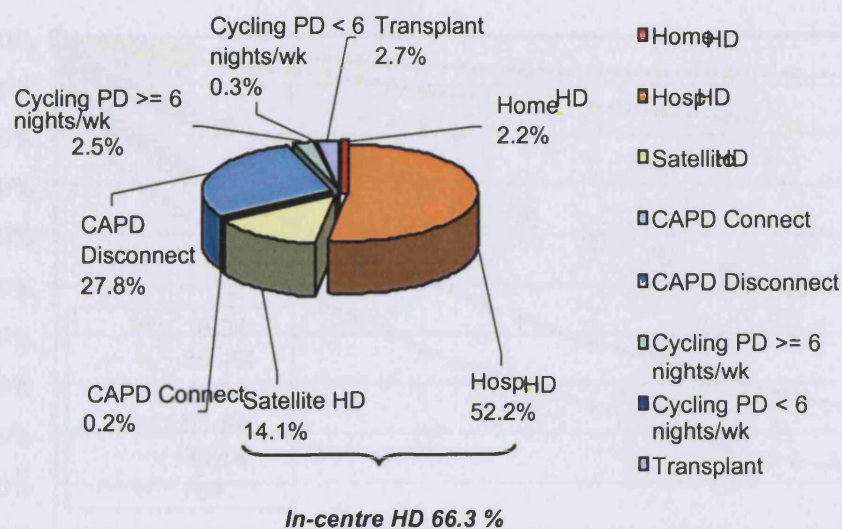


Figure 4 Growth in dialysis patients worldwide
ROW = Rest of world (Gurland Lysaght 1999)

Figure 5 Life expectancy with ESRD is poor despite renal replacement therapy KM survival on renal replacement therapy over six years by age group (UK Renal Registry)

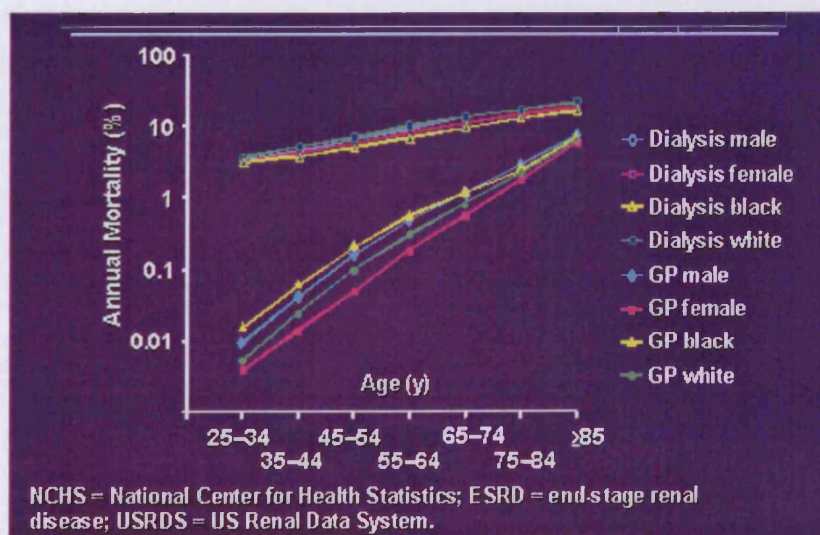
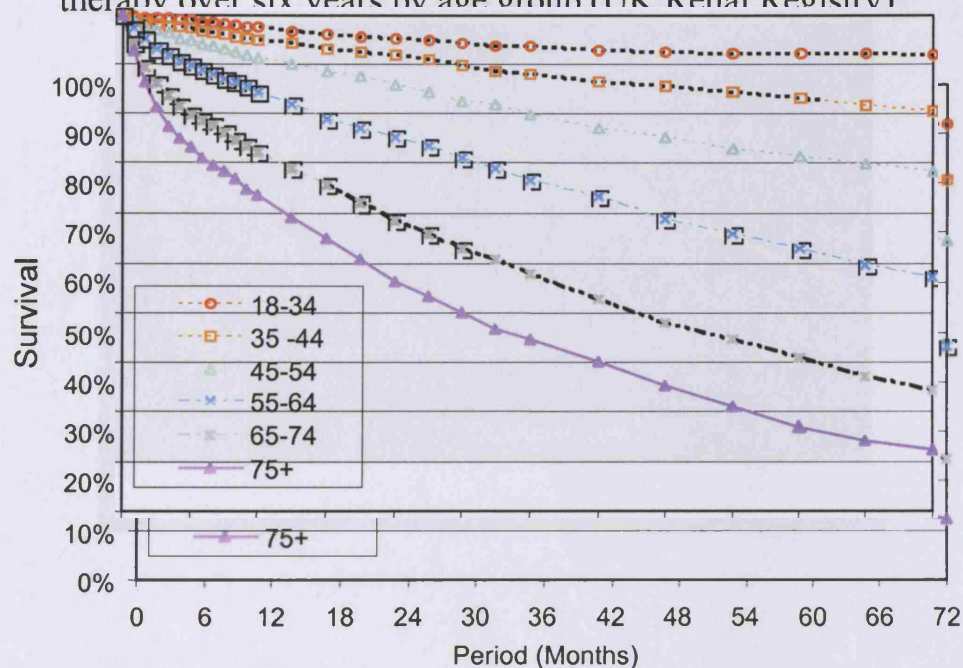


Figure 6 Cardiovascular mortality in the general population (NCHS) and in ESRD treated by Dialysis (USRDS)

Figure 7 Hypertension is a risk factor for mortality
Charra et al Am J Nephrol 1996; 16: 35

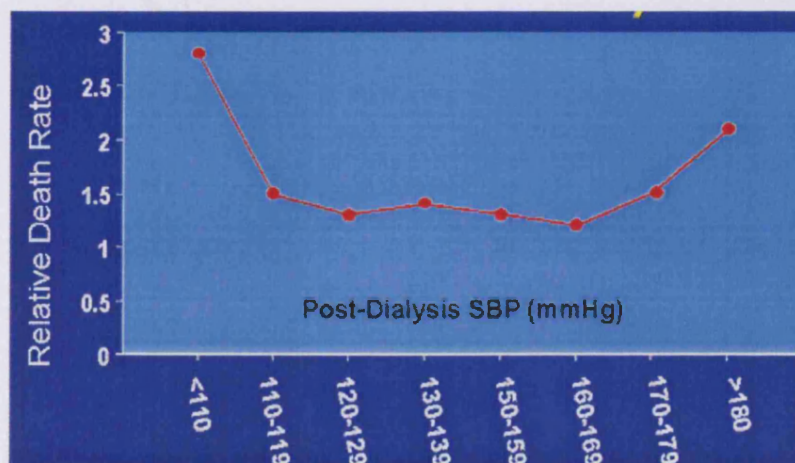
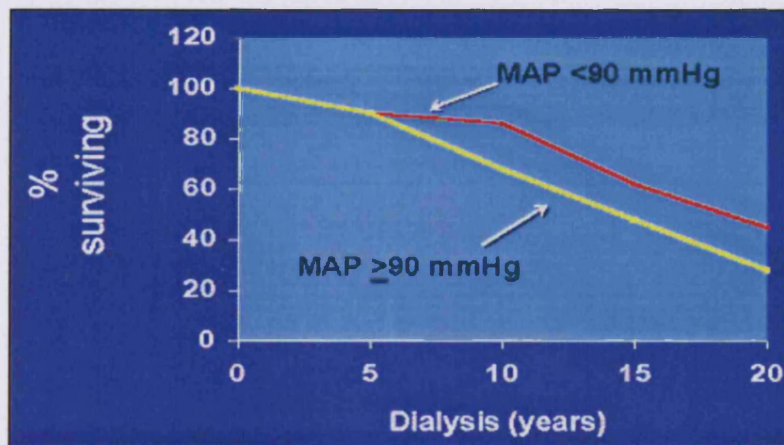


Figure 8 U curve association between BP and mortality
Zager PG et al KI 1998; 54: 561

Figure 9 Dialysis Hypertension in dialysis facilities in a single state in the USA *Salem et al AJKD 1995*

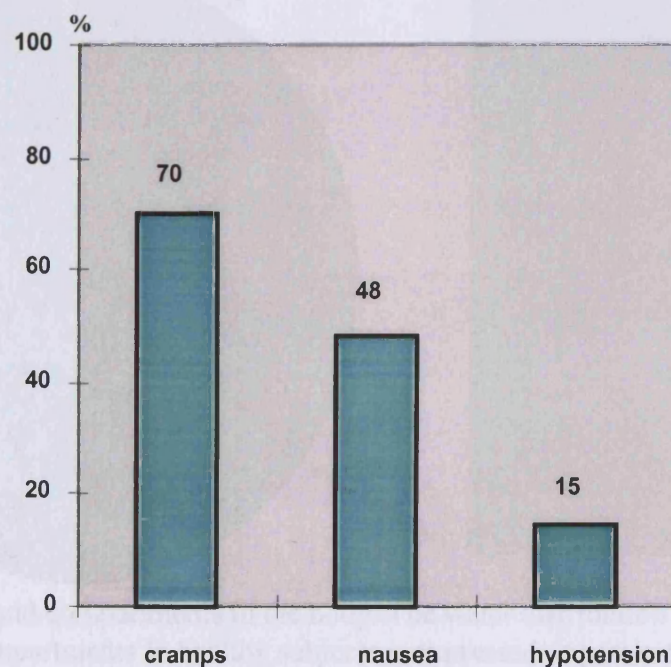
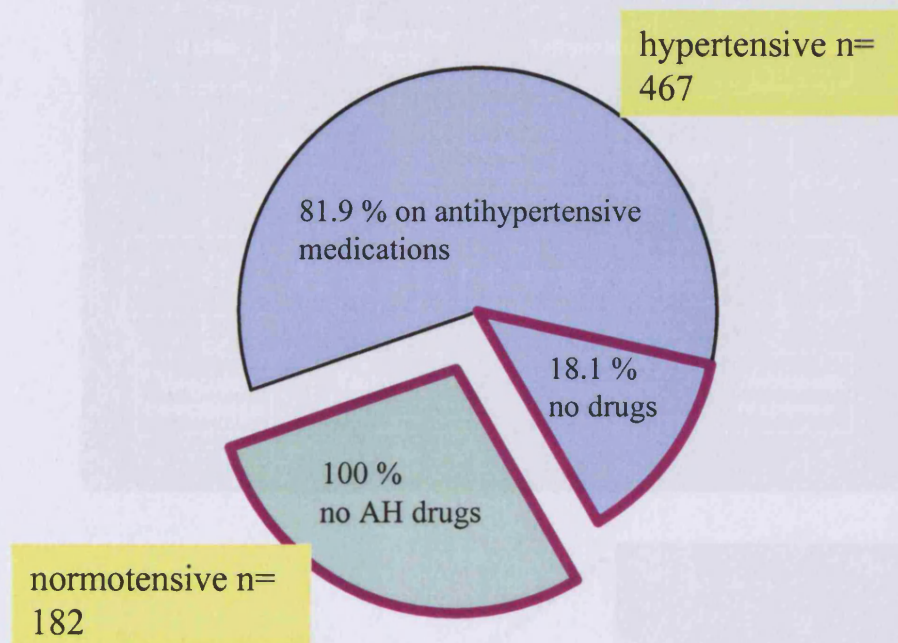


Figure 10 The hypovolemic symptoms are a major cause of premature termination or missed dialysis sessions *Rocco MV et al JASN1993*

Figure 11 The mechanisms leading to hypotension are multifactorial and influenced by dialysis and patient related factors

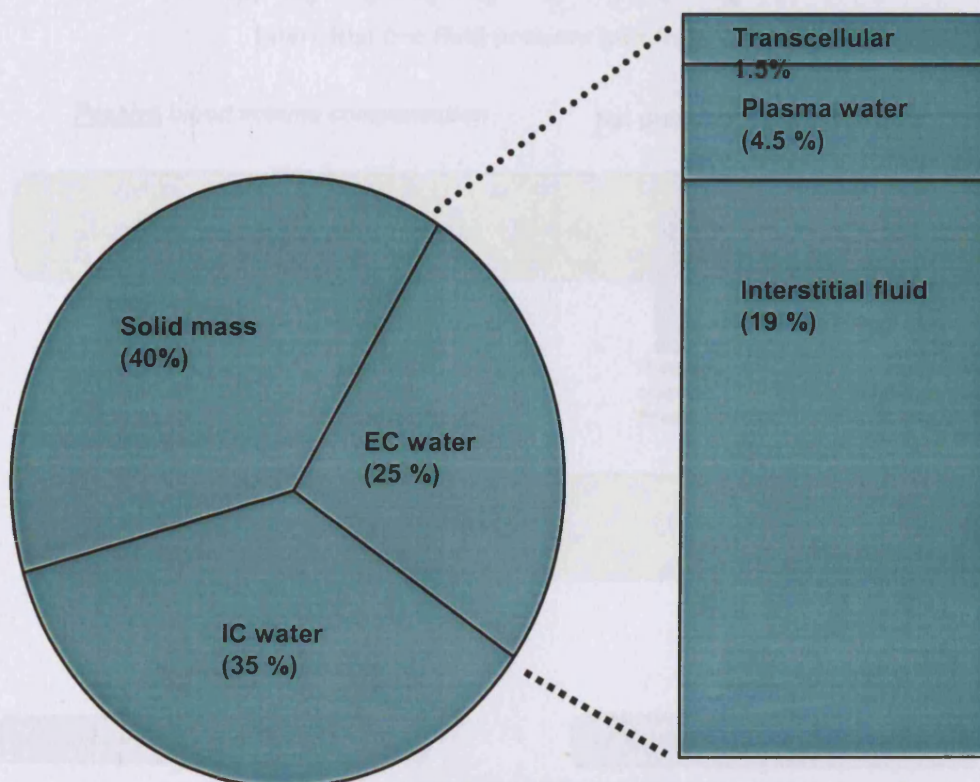
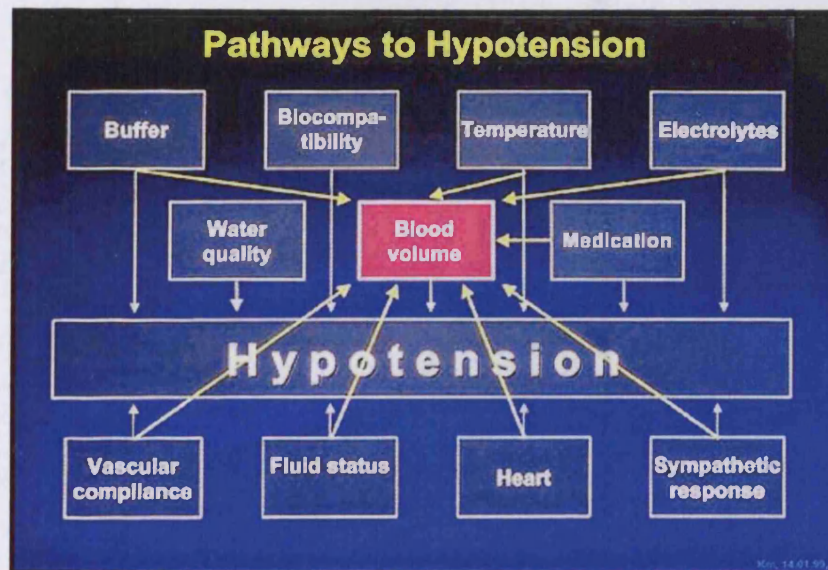
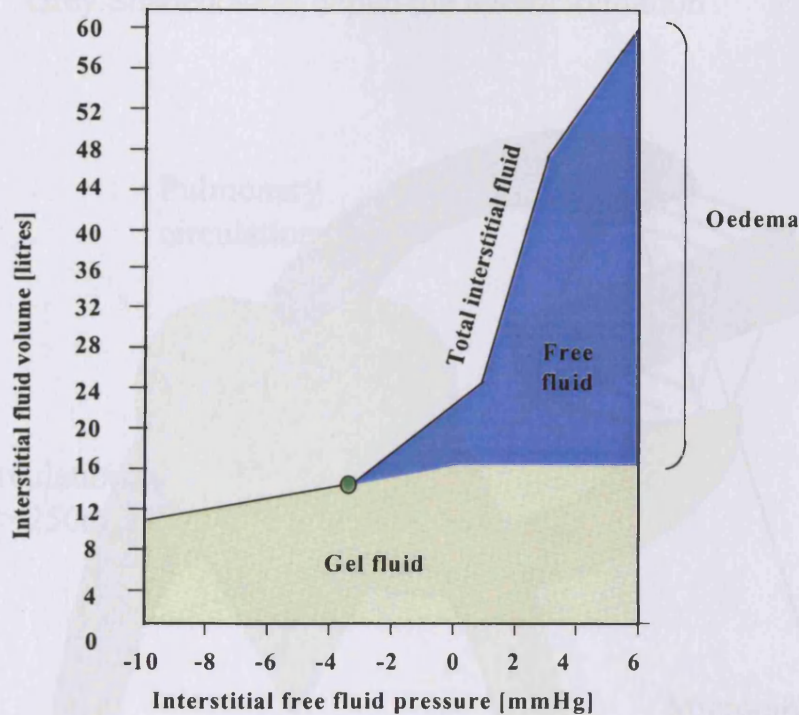


Figure 12 Fluid compartments in the body. The water distribution between the different compartments in healthy subjects is expressed as percentages of total body water (TBW 60%) EC extracellular IC intracellular Ref Despoulos A Silbernagel S; Kidney, salt and water balance. In: Color Atlas of Physiology, 4th ed Stuttgart: Thieme, 1991:139

Figure 13 The interstitial compartment acts as a reservoir for the excess accumulated extracellular fluid



Passive blood volume compensation

$$\text{Net pressure} = (P_c - P_i) - (P_{oc} - P_{oi})$$

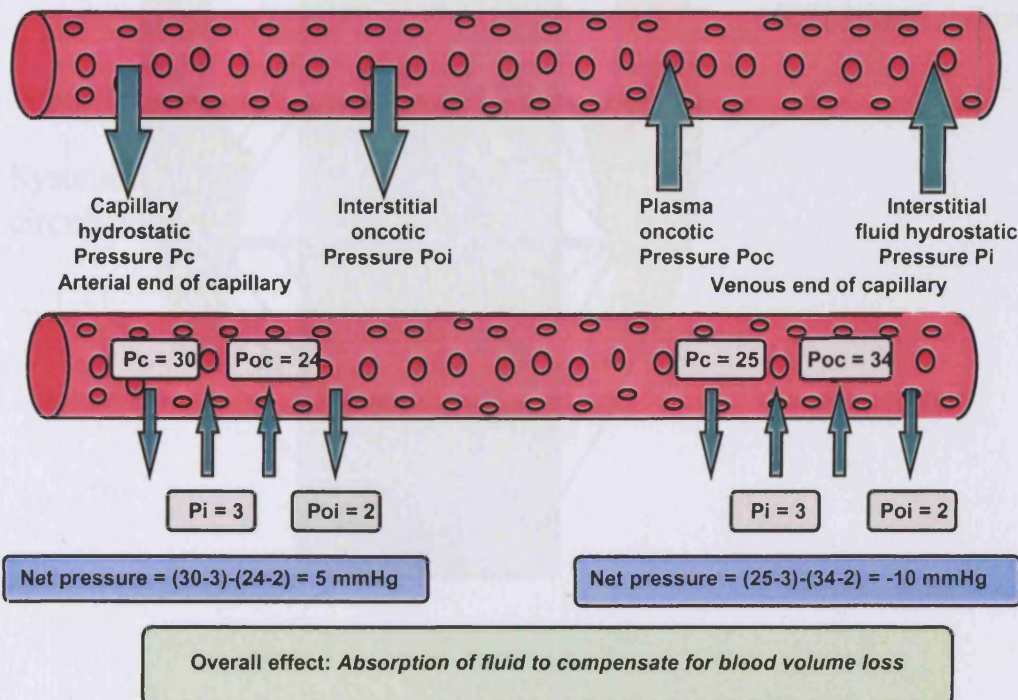
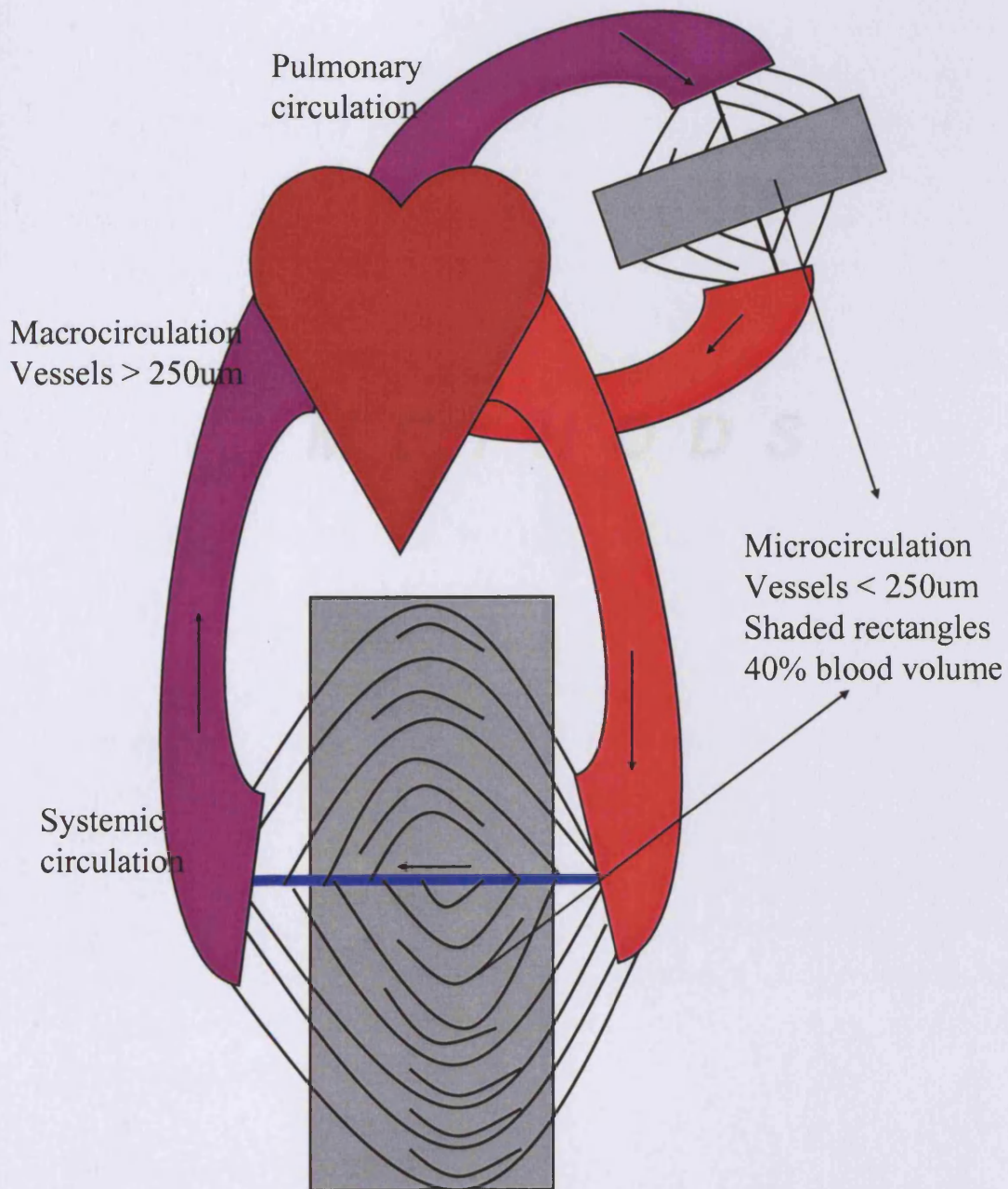


Figure 14 Schematic diagram of the Starling forces governing passive transvascular movement of water between the intravascular and interstitial compartment (a) and the net pressure gradients at arterial and venous end of the capillary (b)

Figure 15 Components of the circulation (schematic)
Grey Shaded areas depict the microcirculation



M E T H O D S

Introduction

Monitoring blood volume responses to ultrafiltration offers many possibilities to deduce some information about fluid shifts. Some pathways to hypotension may be the indirect consequence of fluid removal, emphasising the need for multi parameter data collection. Understanding the more subtle characteristics of blood volume changes also requires additional measurements for more rigorous analysis. The purpose of this chapter is to describe the principle and mechanism of haemodialysis and ultrafiltration methods and the rationale behind selection of a suite of measurement devices, in order to obtain patient specific data of interest during fluid removal. A relatively complex data acquisition system was necessary in order to integrate the measurement devices.

1 The Haemodialysis Procedure

During haemodialysis, diffusion of solutes between the blood and a dialysis solution results in the removal of metabolic waste products and the replenishment of body buffers. Heparinized blood is pumped through a the dialyser on one side of a semipermeable membrane at flow rates of 250 to 550 ml per minute, while dialysate flows in the opposite direction at 500 to 800 ml per minute in order to remove waste products. Resulting urea clearance rates of 200 to 350 ml per minute effect a 65 to 70 percent reduction in the blood urea nitrogen concentration during a three-to-four-hour treatment session; the urea clearance rate also depends on the surface area of the dialyser and the permeability of the membrane. By means of adjustments in the transmembrane pressure across the dialyser, removal of fluid from the plasma into the dialysate can be accurately controlled.

i) Haemodialysis Apparatus

All haemodialysis experiments in this thesis were performed using Fresenius 4008E HD machine (Fig 1) The machine controls and internal fluid pathway (Fig 2) come in contact with the patient via the extracorporeal circuit (Fig 3). All patients received high flux HD 3 times per week using bicarbonate buffer, 1.0–1.4 m² hollow fibre polysulfone membranes (Fresenius HF80) with blood flow rates of 300-500ml/min,

dialysate flow rates 800 ml/min and the Fresenius 2000E delivery system with volumetrically controlled UF [111] The machine was specifically configured to have an inbuilt ultrasonic blood volume monitor, an integrated oscillometric BP monitor and a temperature module. Dialysis was prescribed according to a UKM. [112] Standard dialysate preparations were used for the subjects under study. Dialysate contained Na 138 mmol/l, K 2mmol/l, Ca 1.25mmol/l, Mg 0.5mmol/l, Cl 108.5mmol/l and glucose 5.5 mmol/l, and bicarbonate 35 mmol/l with the conductivity and temperature kept at 140 mS and 36 C. The microbiological quality of the dialysis fluid was monitored as a part of the routine clinical protocol in the unit and consistently achieved recommended standards [113] Careful monitoring of the water-treatment system and the dialysate must be carried out to prevent these adverse reactions. The water quality maintained at the this centre is approximately 10 times better than the minimum recommended and is regularly kept under close surveillance. During the studies there were no incidents related to the water quality standards. All patients had UKM performed 1-3 monthly depending on the level of residual renal function. Using these data, dialysis was individually prescribed to achieve a KT/V 1.2 for thrice weekly dialysed population.

ii) Haemodialysis Membrane

Major advances have occurred in membrane technology with the development of more biocompatible dialyser membranes and of membranes that are thinner and more permeable. High-efficiency dialysers are those with a large surface area that are distinguished by high rates of urea clearance. High-flux dialysers have the additional property of markedly increased hydraulic permeability, which is accompanied by an increase in diffusive permeability, particularly to solutes with molecular weights in the range of 1500 to 5000 — so-called middle molecules. High-flux dialysers have also been defined by rates of clearance of beta₂-microglobulin (molecular weight, 11,800) above 20 ml per minute.

The use of large-surface-area high-efficiency or high-flux dialysers permits high rates of urea clearance to be achieved, allows a concomitant shortening of the time required for dialysis, and offers the theoretical advantage of improved blood

purification by removing the higher-molecular-weight solutes mentioned above. Furthermore, adsorption of molecules, such as beta₂-microglobulin, to the surface of these dialyser membranes constitutes an additional important mechanism of blood purification. [114] Ideally, the haemodialysis membrane should not induce adverse reactions when it comes into contact with the blood; that is, the membrane should be biocompatible. Efforts have been made to minimize reactions by either modifying the cellulose polymer or using noncellulose-based membrane materials. Examples of polymers that can be formulated into more biocompatible membranes include acetate-substituted cellulose and noncellulose-based polyacrylonitrile, polysulfone, and polymethylmethacrylate ; [115] membranes formulated from these polymers may also have the increased permeability discussed above. The membrane structure does not depend on the polymer but on the precipitation step during the manufacturing process.

Membranes used in the study were high flux polysulfone (typically HF 60). High flux membranes are characterised by high diffusive performance (thin layer of 1 µm) and high ultrafiltration coefficient. The membrane itself has high biocompatibility regardless of the membrane permeability. This is due to the following factors a chemical structure free of hydroxyl groups responsible for complement activation and hypersensitivity reactions.

- * an electrically neutral polymer, hence no positive charges which may cause thrombogenicity, no negative charges which can cause anaphylactoid reactions
- an asymmetrical wall structure with hydrophobic domains, which contributes to retention of endotoxins

iii) Access for Dialysis

Obtaining and maintaining adequate access to the circulation remains a major impediment to the long-term success of haemodialysis. The fistula, conduit, or catheter through which blood is obtained for haemodialysis is often referred to as a "dialysis access." The placement of large needles (typically 15 gauge) is required to remove blood and to return it after it has passed through the dialyser. A large, thick-walled fistula can be created by shunting blood from an artery to a vein; the result is the growth and thickening of the venous wall, which then tolerates repeated

cannulation. The Cimino–Brescia fistula, in which the cephalic vein is anastomosed to the radial artery is preferred because its higher survival rate.[116] All studies were performed using functioning AV fistula or graft with less than 10% recirculation. When dialysis is urgently required, a double-lumen dialysis catheter is used. Implantation of a dual-lumen cuffed catheter is a good option for patients who have delayed recovery from acute renal failure, who require access for dialysis until a fistula matures, or who lack any other suitable site for graft placement. If carefully maintained, almost half of these catheters remain functional at one year.

2 ULTRAFILTRATION

i) Principle and Theoretical background

Removal of excess body water is an important function of both the artificial kidney and peritoneal dialysis. Patients with chronic renal failure requiring treatment with the artificial kidney will in most instances require reduction of their total body water in conjunction with their dialysis. The process of removing fluid by convection is known as **ultrafiltration**. For each dialysis a judgment must be rendered as to how much fluid should be removed during treatment to prescribe ultrafiltration. This presupposes that two pieces of information are available to the clinical investigator: 1) the degree of deviation from the proper total body water content of the patient under consideration and 2) the ultrafiltration rate of the artificial kidney to be used or of the peritoneal membrane. This section deals with the practical and theoretical aspects of ultrafiltration across the artificial kidney.

Ultrafiltration across the peritoneal membrane must be accomplished osmotically. Glucose, sorbitol, mannitol and other osmotically active solutes have been used to create the pressure gradient. Commercially available dialysis solutions most often offer a 1.5 g/dl and 4.25 g/dl glucose solution. There is considerable variability from patient to patient on the extra amount returned by ultrafiltration. An explanation may well be that the membrane involved is biological and subject to differences between patients as well as changes with time in the same patient. With a duration of each exchange of 30 to 70 min and a production of 500 ml of ultrafiltrate per exchange an ultrafiltration rate of 7 to 16 ml/min is accomplished. These figures are somewhat

below those for extracorporeal dialysis where ultrafiltration rates of upto 35 ml/min may be easily obtained with a standard dialysis membrane. It should be noted that the vascular volume during ultrafiltration is dependent on the comparative rates of movement of fluid leaving the vascular space by ultrafiltration and entering the vascular compartment from interstitial and possibly intracellular sources. The rate of mobilization of extravascular fluid is specific to the individual. Fluid sequestered in body cavities such as in the pleural space or in the pericardium usually is recruited very slowly and may take weeks or months of repeated efforts to remove even in the absence of any identifiable cause for local fluid accumulation like active pleuritis or pericarditis.

Silverstein et al [117] have also noted that fluid removal conducted without dialysis using an ultrafiltration cell (0.2 m² area) caused few if any symptoms in maintenance dialysis patients presenting with an acute fluid overload. This observation holds for the use of this device in series with conventional haemodialysis equipment. For reasons that are not clear, patients ultrafiltered in this manner have fewer symptoms. The process of Ultrafiltration is dependent on 4 key factors: ultrafiltration coefficient, hydrostatic and oncotic gradients, osmotic gradient with glucose and reflection coefficient.

Membrane packages using hollow fiber format (used in the current experiments) in general do not have an obligatory ultrafiltration rate, as the blood path resistance to flow is low. In these cases dialysis fluid is often pulled through the dialyser rather than pushed and a hydrostatic pressure for ultrafiltration is achieved by the dialysis fluid pump pulling against a partially occluded (by an adjustable clamp) inflowline and (or) the blood pump pumping against a partially occluded blood return line. Resistance to flow in the unclamped dialysate path is sufficiently low so that at satisfactory flow rates (200 to 500 ml/min) little, if any, obligatory ultrafiltration occurs.

Theoretical background

When a concentration gradient exists across a semipermeable membrane, both solute and water tend to move in a direction to discharge that gradient with time. Solute and water move in opposite directions across the membrane to achieve equilibration. The gradient may be expressed in terms emphasizing the solute (e.g., number of

mg/dl of solute on side A, minus number of mg/dl of solute on side B) or in terms emphasizing the water (e.g., number of milliosmoles on side A, minus number of milliosmoles on side B). Osmolality is generally considered as the number of solute particles per kilogram of water. Alternatively, it may be considered as a measure of the number of water molecules per kilogram of solution i.e., the "concentration of water". There are two ways in which a concentration gradient for water may be achieved: osmotically and hydrostatically. For artificial kidney membranes both osmotic and hydrostatic (hydraulic) force may be used to achieve the concentration gradient necessary to cause ultrafiltration (separation of macromolecular solutes, i.e., protein and cellular elements from plasma water). The hydrostatic force is the more effective of the two techniques. The hydrostatic force across the peritoneal membrane remains rather constant and hence osmotic force is all that is available to achieve increased ultrafiltration.

An equation that relates ultrafiltration rate to these forces is frequently written [118]

$$J_f = Q_f / A = LP (\Delta P + \Delta \pi)$$

Ultrafiltration rate per unit area of membrane = (permeability of membrane to water) x (hydrostatic force + osmotic force) where

J_f = the volume flux rate per unit membrane area across the membrane for water (ml/min/ cm^2), usually *negative* as water is lost from the subject.

LP = the permeability of the membrane for water, i.e., the volumetric flow rate of water per unit area of membrane per unit pressure gradient (ml/min. cm^2 . mm Hg),

Q_f = flow rate of ultrafiltrate (ml/min), usually negative as flow is away from the patient

A = Area of the membrane (cm^2),

ΔP = The hydraulic pressure gradient from blood path to dialysis fluid path (mm Hg),

$\Delta \pi$ = The osmotic pressure gradient from blood path to dialysis fluid path (mm Hg); $\Delta \pi$ is frequently measured in mOsm/l and may be converted to mm Hg using $1.0 \text{ mosm} \simeq 19 \text{ mm Hg}$.

The hydrostatic and osmotic forces are summed in this equation, since with dialysis, using either the peritoneal or a synthetic membrane there is a deliberate osmotic

gradient favoring water movement from blood to dialysate. It should be noted that where isotonic dialysis fluid is used the osmotic pressure provided by the plasma proteins favours water movement from the dialysis solution to blood and the contribution of $\Delta \pi$ to J_f is reversed.

Water rarely if ever moves by single molecule diffusion, but rather by "bulk" flow in which movement of "blocks" of water occur much as you would consider the movement of pure water in a pipe when an inlet to outlet pressure gradient is applied. It is apparent that in subsequent instants this rather straightforward conceptualisation becomes much more complicated. To cite some of the events, glucose is small enough to move by diffusion down its concentration gradient from B to A reducing the osmotic driving force; further, the water arriving on side B dilutes the glucose concentration adjacent to the membrane lengthening the diffusion path over which the concentration gradients apply and slowing their discharge.

Further, protein, a macromolecular structure present in solution on the blood side cannot cross the membrane and will exert an osmotic effect (oncotic pressure) favouring water reabsorption from the dialysis bath. Finally, the distribution of charges on the solute particles and the requirement for electroneutrality across the membrane will modulate the movement of electrolytes in response to their concentration gradient.

The convecting of solutes along with water through a membrane in response to a pressure gradient (osmotic or hydrostatic) is termed "solvent drag". When the membrane exerts no restraining force (sieving effect) on the solute, i.e., the solution does not change in concentration as it traverses the membrane, then the terms "bulk", "plug" or "Poissilian" flow describe the events.

The ultrafiltration rate in the human glomerulus and capillary beds such as the splanchnic circulation are dominated by considerations of oncotic pressure. The importance of the oncotic pressure lies not in its magnitude but in the nearly equal hydrostatic pressure across the capillary wall. By contrast in the artificial kidney hydraulic pressure (ΔP) almost invariably exceeds oncotic pressure. Small solutes other than protein, present in the dialysis fluid or uraemia plasma (glucose, urea) contribute significantly to the osmolality of the solution but because of their low reflection coefficient result in comparatively little water flux across the membrane.

ii) Mechanics of Ultrafiltration

Since blood is pumped through a dialyser fibres under pressure, it is important to make sure that the hydraulic pressure of the dialysis fluid balances the blood pressure in the dialyser fibre. If a pressure balance is not achieved fluid will be transferred either from the blood to the dialysate or vice versa. The difference in the pressure between the blood and dialysate compartments is called the *transmembrane pressure (TMP)* and is defined as the venous pressure - the dialysate pressure ($P_v - P_d$). It is not possible to measure the pressure in the dialyser directly, so the venous pressure is used instead for the purposes of calculating the TMP.

It is usually necessary to remove fluid from a patient by ultrafiltration. To provide ultrafiltration a pump removes a precise volume of fluid from the dialysate. This has the effect of reducing the dialysate pressure so that P_d is less than P_v . Hence ultrafiltration generates a positive TMP. Since the TMP is positive, there is a transfer of fluid from the blood to the dialysate thus reducing the patient's blood volume. The ease with which fluid can be pulled across the membrane by ultrafiltration is specified by the ultrafiltration coefficient K_{UF} of the dialyser. (Fig 4) It is simply the amount of TMP that needs to be applied across the membrane to provide a given

flow rate through the membrane (UFR): $K_{UF} = \frac{TMP}{UFR}$

Modern dialysis machines that use a volume-sensitive ultrafiltration device (volumetric UF) have been useful in providing an even and highly regulated decline in plasma volume during dialysis. [111]

Early machines employed a single effluent pump drawing dialysis fluid through the dialyser via an upstream throttle valve, which produce a degree of negative pressure in the fluid compartments of the dialyser. The flat plate configuration prevented any backfiltration. Ultrafiltration, the removal of water from the plasma by hydrostatic force was self regulatory, the negative pressure being resisted by rising oncotic pressure as the blood volume was reduced. Volumetric control of ultrafiltration was introduced in the early 1980s. [111] The amount of dialysis fluid entering or leaving the dialyser is rigidly controlled by the diaphragm/fixed displacement sensor systems

that monitor flow rates in the afferent and efferent fluid streams using an electromagnetic technique. The amount of UF required is preset by the operator. The direct control of fluid flux removes the potential for physiologic self-regulation. The volumetric machines have now become standard.

During pilot studies preliminary checks were undertaken to ensure accuracy of the calibrated volumetric device by an hourly collection from the ultrafiltrate port in a measuring cylinder. There was an approximate error of 18 ± 4 mls of collected ultrafiltrate for each litre recorded on the calibration pump.

iii) Ultrafiltration Profiling

Principle of Perturbation analysis

In order to characterise patient responses to fluid removal, an intermittent ultrafiltration profile provided a suitable perturbation. This method elicits rapid changes in blood volume causing various compensatory mechanisms for blood volume and pressure to be invoked. Intermittent profiles have been applied previously in patients. In complicated systems an appropriate method by which the dynamics of the system may be characterised involves periodic application of a perturbation. A technique called "system identification" has been applied in the industrial setting for some years in situations where it is particularly difficult to model a very complicated system. This method involves the application of a suitable test signal from which the output may be monitored from suitable sensors (Fig 5)

A smooth exponential rise could be approximated with a single or set of 1st order differential equations. By the same token, an oscillatory response could be modeled by an under damped second order system. Beyond this, higher order systems become considerably more difficult to characterise and the process of system identification rapidly becomes an area of specialized analysis. The advantage of system identification is that it allows the dynamics of the system to be investigated at periodic intervals, which caters for changing parameters. The test input allows certain parameters to be obtained which may then be considered constant over a limited range of time. When ultrafiltration is initiated a number of physiological compensatory mechanisms are

involved both for blood volume regulation and BP maintenance. In this sense characterisation of an individual patient response can be difficult since the patient may be regarded as a complex arrangement of dynamic elements linked by a highly integrated array of regulatory mechanisms.

Intermittent Ultrafiltration Profile

In order to characterise patient responses to fluid removal; a high fixed rate intermittent ultrafiltration profile 2-3l/hr following an isovolemic period of 30 mins with intervening rest periods provided a suitable perturbation. This method generally elicits rapid changes in blood volume causing various compensatory mechanisms for blood volume and pressure to be evoked. Intermittent profiles have been previously applied in patients on haemodialysis. The principle of intermittent ultrafiltration to extract information about refilling has been demonstrated by Stiller and Mann [120] from which some empirical relationships could be derived.

Two types of ultrafiltration profile used in the following experiments are described below

PROTOCOL A (Fig 6a) Variable volume Ultrafiltration

The volume of fluid removed was graded during dialysis. Using a fixed ultrafiltration rate 3l/hr the volume removed in the initial bolus was 40% of the desired total UF volume. This was preceded by an isovolemic period of 20 –30 mins. The volume of fluid removed in the subsequent pulses was linearly reduced using 30%, 20% and 10% of UF for rest of the pulses. The ultrafiltration bolus and the rest time was fixed. By this method the ultrafiltration stress applied to the patient by the end of the treatment was reduced in a gradual manner as dry weight was approached.

PROTOCOL B (Fig 6b)

Using a fixed ultrafiltration rate, following an initial isovolemic period, an initial bolus of 40 % of the total prescribed ultrafiltrate volume was removed. Subsequently 20% of the UF volume was removed in equal steps each followed by an intervening UF free period for blood volume recovery.

3 Measurement of relative blood volume

i) Biosensors

In terms of instrumentation, a sensor is defined as a measuring device that exhibits characteristic of an electrical nature when it is subjected to a phenomenon that is not electric. Under this definition a sensor could be regarded as a transducer since it is a system that transforms one physical quantity into another, which is a function of the first.

In a biosensor, a phenomenon is recognised by a biological system called a bioreceptor, which is in contact with the sample and forms a sensitive component of the biosensor. In order to measure a physical quantity, a sensor must fulfil a number of criteria. The measurements itself must have repeatability, reproducibility, selectivity, sensitivity, a linear region of response and a good response time (time taken to reach a steady state). [121]

A biosensor can be considered as a combination of a bioreceptor and a transducer. Commonly used biosensors today are enzymes, microbiological, piezoelectric crystal etc.

Ultrasonic Flowmeters

Small magnitude pressure disturbances are propagated through a fluid at a definite velocity (speed of sound) relative to the fluid. The term ultrasonic refer to the fact that in the practice the pressure disturbances are short bursts of sine waves whose frequency is above the range audible to human hearing about 20000 Hz. A typical frequency is 10 Mz. A common approach is to utilise a piezoelectric crystal transducer as transmitter and receivers of acoustic energy. In a transmitter electrical energy in the form of a short burst of high frequency voltage is applied to a crystal, causing it to vibrate. If the crystal is in contact with fluid, the vibration sense will be communicated to the fluid and propagated through it. The receiver crystal is exposed to this pressure and responds by vibrating. (Fig 7) The vibratory motion produces a signal in proportion, according to the usual action of piezoelectric displacement transducers. These can be used as ultrasonic density measuring technique. The

crystal transducer serves as an acoustic impedance detector. Acoustic impedance depends upon density of speed and sound. Since a signal proportional to the speed of sound is available division of this signal into the acoustic impedance signal gives a density signal. The attenuation depends on the density of the material through which the signal passes. [121]

ii) Relative blood volume monitoring by ultrasonic method

During the last decade there has been considerable interest in the development of non-invasive extracorporeal methods for the determination of relative blood volume. This has been made possible by advances in the development of biosensors. The RBV is the ratio of the current blood volume to the initial blood volume at the start of treatment. $RBV_t = C(0)/C(t)$ and can be expressed in percentages where C is the time dependent concentration of the blood components e.g. cells, protein, haemoglobin. All techniques rely on the fact that the solid blood components remain confined to the vascular space. As plasma water is removed the concentration of these components increase. Preliminary algorithms for controlling blood volume depending on patient specific criteria have been applied demonstrating a reduction in morbid events such as dizziness, nausea or symptomatic hypotension.

A number of methods have been developed for blood volume monitoring including optical, ultrasonic, electrical and mechanical methods. The ultrasonic technique initially devised by Schneditz [122,123] has been adopted for several reasons, among these is the availability of high precision transient time measurement technology and the low costs of disposable materials and high achievable precision of RBV measurements. [124]

Selection of a blood volume monitor (BVM)

Since the blood volume monitor was the primary focus of the current research, accurate determination of relative blood volume was a prerequisite to the entire programme of research involving patient studies. It was likely that a range of blood chemistries would be encountered across the patient study group. Therefore, preliminary research was undertaken in this unit in a number of blood volume

monitors (BVMs) employing different measurement principles in order to investigate artefacts influencing the measurement of blood volume. The BVMs evaluated involved a Critline (optical), [125,126] Fresenius (Ultrasonic) and an in-house (optical) BVMs. A set of in-vivo and in-vitro experiments was performed in which the effects of changing haematocrit, protein and sodium concentration were quantified. The main findings of these studies were as follows:

- Both plasma protein and sodium changes have a major influence in the precision of relative blood volume measurements in BVMs employing optical methods.
- Appropriate correction algorithms are necessary in optical BVMs in order to compensate for light scattering effects.
- Blood flow rate is a major source of disturbance in the in-house BVM.

The Fresenius ultrasonic BVM [127] consistently demonstrated superior performance when compared with the other BVMs evaluated. Consequently, the ultrasonic Fresenius BVM was the device of choice for the current research reported in this thesis.

Ultrasonic measurement of blood volume

Measurement of blood density during haemodialysis in order to calculate blood volume changes was undertaken nearly 25 years ago by Holzer et Al [128] During the late 1980's Schneditz [123] developed a new method for measurement of blood volume using a disposable polymer tube which could be inserted in series with arterial blood tubing.

Principle of operation

The ultrasonic or speed of sound technology measures the total protein concentration which is the sum of the plasma protein and the haemoglobin in red cells. Since these two components make up the majority of the solid component of blood then the velocity of sound in blood is predominantly dependent on total protein concentration.

Short acoustic pulses at 3mhz are transmitted through the disposable cuvette, which is machined accurately to narrow tolerances, in order to ensure a fixed transmission distance. The transit times of these pulses are then measured accurately to compute the velocity of sound through the medium. Since the velocity of sound in temperature dependent, a thermistor continually monitors the blood temperature. In addition a Peltier element is integrated into the sensor assembly in order to minimise the interference from environmental temperature fluctuations.

During normal operation the system is calibrated with isotonic saline. The ratio of the velocity sound in water (saline) to the velocity of sound in blood is then related to the blood water concentration and the relative blood volume may be calculated.

The ultrasonic technique exploits the principle that sound speed in blood depends on the total protein concentration, the sum of plasma proteins and haemoglobin. Changes in sound speed can be related to changes in total protein concentration. The relative blood volume may be determined from the protein concentration.

A polycarbonate measuring cuvette is located in the arterial line of the blood extracorporeal circuit before the pump as shown in the figure. Blood passes through the cuvette from the bottom to the top, which is slightly inclined rather than vertically placed thereby avoiding accumulation of air bubbles. (Fig 8) An ultrasonic pulse is transmitted through the cuvette containing the blood. A silicon rubber insert ensures sound coupling to the measuring cuvette. (Fig 9) The transit time of the pulse, which is dependent on the speed of sound, through the blood is measured by the BVM. A high precision temperature measurement ($<0.1^{\circ}\text{C}$) is required to compensate for the dependence of sound velocity on blood temperature. An empirical function found by Schneditz is used to derive total protein concentration (TPC) from sound velocity and temperature. [124] Using the principle of mass conservation, the RBV can be determined from TPC as function of time. Haematocrit and haemoglobin can be calculated from TPC by simple linear equations assuming a mean plasma protein concentration of 72.5 g/l at the start of treatment. During the priming procedure when the circulating saline is in the extracorporeal circuit has reached a stable temperature, the BVM performs an automatic calibration. On detecting the presence of blood, the measurement of relative blood volume commences as soon as a sufficient stable temperature and sound velocity is reached,

typically after 1-3 min. In addition to the measurement of RBV, it also measures haematocrit and haemoglobin.

Validation of method

In validating the device, the measurement of RBV, reference photometer method provided the haemoglobin measurements for comparison with the BVM. [127] Haematocrit was determined by the microcentrifuge. The photometric cyanmethaemoglobin method is regarded as the most accurate standard by which haemoglobin standard may be determined. 10uL of whole blood was withdrawn to a minicuvette manually with a pipette and then mixed with a reagent of hexacyanoferrate and potassium cyanide. This procedure lyses red cells, eliminating light scattering effects. Haemoglobin was determined by Dr Lange Miniphotometer LP2, which has a numerical resolution of 0.1g/dl. Haematocrit was measured from whole blood taken from arterial lines and measured using heparin coated microcentrifuge capillaries.

The RBV measured by BVM and that determined by the photometer showed excellent correlation $r=0.96$ $n = 882$. The mean error between the two methods was 0.07%, SD 1.7% demonstrating high accuracy and reproducibility of the BVM. No dependencies were observed for on varying ultrafiltration rates. The noise on the RBV signal is very low ($<0.2\%$, sampling rate 10s). [125]

The correlation between the Hct by microcentrifuge and BVM showed a significant correlation of $r = 0.88$. The mean deviation was -0.55 Hct and SD 2.9%. The data from BVM was analysed for sensitivity to different blood compositions.

Infusion of 200ml saline into a patient with a blood volume of 5000mls represents a 4% change in blood volume. In order to resolve, a blood volume sensor should provide an accuracy of 2%. The results of the evaluation suggest that the BVM has a SD of 1.7%, which satisfies the measurement criteria for routine blood volume monitoring. A clear RBV signal with minimal noise is necessary in order to assure a rapid detection of sudden blood volume changes. The quality of the signal is a precondition for using the BVM for automatic blood volume control. A decrease in the plasma osmolality can lead to a swelling of erythrocytes, which would not be detected by BVM. Hence BVM measures an osmolality corrected haematocrit.

iii) Other techniques for non-invasive extracorporeal blood volume measurement

OPTICAL BVMs - PRINCIPLES

Considerable research effort has been directed towards the use of optics to measure haemoglobin and oxygen saturation in whole blood. In a homogenous medium such as haemolysed blood the absorption of light is dependent on the composition of the medium, the optical path length and the wavelength of light. The attenuation may be described simply by the Lambert-Beer law. Light passing through a suspension such as whole blood, is attenuated as a result of scattering and absorption effects. Scattering is caused by a combination of reflection, diffraction and refraction where the red blood cell is large by comparison with the wavelength of light used in measurement applications. An electromagnetic wave theory developed by Twersky and a photon dilution theory by Zdrojkowski have been proposed to explain the attenuation of light in whole blood. [129] Later Anderson and Sekelj used an integrating sphere photospectrometer to demonstrate that total optical density of blood could be expressed as the sum of absorption and scattering components as predicted by Twersky. [129] Most commercial devices based on optical transmission of light operate in the linear region, which conveniently covers the range of haematocrits likely to be encountered in routine clinical practice. In this range total optical density is proportional to absorbed light only and the Lambert-Beer theory is valid.

The absorption characteristics of whole blood depend additionally on the oxygen content for a given wavelength. Mendelson and Cheung showed that the absorption spectrum of de-oxyhaemoglobin (Hb) and oxyhaemoglobin (HbO₂) were equivalent at a wavelength close to 800 nm known as the isobestic point of blood. In practical applications involving LEDs as inexpensive sources of relatively monochromatic light, wavelengths of 800 nm are not available, so it is usual to use a dual wavelength system. One LED is selected close to the isobestic point, usually an infrared wavelength at 820 nm. A second LED in the visible spectrum is used where the sensitivity to HbO₂ is greatest to correct the infrared measurement for changes in blood oxygenation.

Optical transmission BVMs

The first optical BVM was developed by Wilkinson et al. [130] The device was designed to clip around standard PVC blood tubing, held firmly in place by polished glass inserts, to ensure a relatively constant optical path length. The BVM was later modified by Aldridge [130] to include a second channel to correct for changes in oxygen saturation. A variant of this device was later developed by Mancini the basis of a dialysis machine integrated system available commercially, the HemoscanTM. [131] Steuer evaluated the use of a multiple wavelength system (including oxygen saturation correction) for determination of haematocrit. This technology was further developed for the production of the CritlineTM blood volume monitor. [126, 132]

Optical reflectance BVMs

Some blood volume monitor implementations detect the light, which is back scattered. Typically the LED emitter and photodiode are mounted immediately adjacent to each other. By contrast with the transmission systems, this method has the advantage that a fixed optical path length does not have to be established. DeVries [133] constructed a device to clip onto standard blood tubing producing a reasonably linear relationship between reflected light and haemoglobin concentration in response to fluid removal.

More elaborate devices have been developed in which the LED and photodiode have been integrated into one hybrid device. The main motivation behind such efforts has been the application for implantable devices used to monitor both haematocrit and blood oxygenation. As a result of this technology there has been the need to consider more carefully factors such as the separation between the source and detector and the selection of wavelength.

Haemoglobinometry

Schallenberg et al. [134] describe a system involving a continuous withdrawal of blood into a secondary extracorporeal circuit. Dilution with hypotonic ammonia

solution causes haemolysis liberating haemoglobin. This process converts haemoglobin almost entirely into the oxygenated form, which may be measured accurately from the optical absorbance through a fixed path length cuvette.

Reasonable accuracy could be assumed with this method since the optical difficulties encountered with scattering in whole blood are eliminated, although there is no evidence in the literature of validation studies. In practice this technique is unsuitable for routine use due to the requirement for considerable extra hardware. Since there is an inherent transport delay involved in the dilution process, the transient response is slow which may limit the usefulness of this method in the detection of rapid changes in blood volume.

Conductivity

The electrical impedance of whole blood is frequency dependent. At low frequencies current flows only through the extracellular spaces (plasma) since the reactance of the red cell membrane is high. The conductivity of the blood is then dependent on the ionic composition and temperature of the plasma for given volume element. [135] At high frequencies the reactance of the cell membrane becomes small and current passes through both the intracellular and extracellular spaces. Consequently the conductivity of the blood increases. The resistive components of the blood in the intra and extracellular compartments may thus be determined through impedance and phase shift measurements at two or more frequencies. The relative difference between the resistances in each compartment provides a measure of the concentration of haematocrit per unit volume of blood, hence the relative blood volume. In theory, the influence of electrolyte variations should be minimal since cells generally maintain electrical equilibrium across the cell membrane. [135]

Viscometry

Removing water from blood causes the viscosity to increase, as there is a greater concentration of solid per unit volume of blood. Greenwood et al. [136] constructed a modified extracorporeal circuit to divert a continuous flow of blood through a

borosilicate capillary tube. The dynamic pressure drop across the capillary was measured with suitable pressure transducers.

The change in pressure drop caused by viscosity increase reflected the change in blood volume. This method suffered from the problems incurred due to variable flow conditions in the extracorporeal circuit. In addition the relationship between relative viscosity and blood volume was found to be patient specific, depending on the haematocrit and plasma protein concentration.

The first continuous blood volume monitor was developed over 15 years ago by Stiller and Mann using a single frequency of 400 Hz. [134] Other authors have since devised sensor systems for impedance monitoring of whole blood, some including a thermistor in order to provide temperature compensation. [137] A configuration of circular electrodes is generally used made either from stainless steel or platinum. The De Vries system [135] consisted of two outer electrodes for injection of current and an additional pair of inner electrodes for impedance and phase measurement. Conductivity based blood volume monitors have yet to be considered for commercial development. A few questions remain concerning the accuracy of the prototypes and improved performance over other types of blood volume monitor would need to be demonstrated. In addition a disposable electrode system is unlikely to be particularly cost effective.

4 *Blood and surface Temperature*

The blood temperature module (Fresenius BTM) was used to monitor changes in core temperature during dialysis. The device consists of two sensor heads through which the arterial and venous blood tubing is inserted. Fig 10 [138, 139] The device allows continuous monitoring of the venous blood temperature at the arterial end as it leaves the patients' fistula (core temperature) and the temperature of the blood as it re-enters the circulation. The dialyser is a very efficient heat exchanger. Changes in the temperature of the incoming dialysis fluid are rapidly equilibrated with the venous blood returning to the patient. Warming of a patient's blood can occur both through vasoconstriction and an inappropriately high dialysate temperature. A thermal probe applied to the skin surface over the forehead measured skin temperature.

5 Haematocrit measurement

Volume of Packed Red Cells (Haematocrit)

The volume of packed red cells (VPRC), or haematocrit, is the proportion of the volume of a blood sample that is occupied by red blood cells. The haematocrit may be determined manually by centrifugation of blood at a given speed and time in a standardized glass tube with a uniform bore, as was originally described by Wintrobe [140]. The height of the column of red cells compared with that of the total column of blood following centrifugation yields the haematocrit. Both macro (using 3-nm test tubes) methods with low-speed centrifugation or micro methods using capillary tubes and high-speed centrifugation may be used.

The manual method of measuring haematocrit has proved to be an accurate method of assessing red cell status. It is easily performed with little specialized equipment, allowing it to be adapted for situations where automated cell analysis is not readily available, or for office use. However, several sources of error are inherent in the technique. The spun haematocrit measures the red cell concentration, not red cell mass. Therefore, patients in shock or with volume depletion may have normal or high haematocrit measurements due to haemoconcentration, despite a decreased red cell mass. Technical sources of error in manual haematocrit usually arise from inappropriate concentrations of anticoagulants, poor mixing of samples, or insufficient centrifugation. Another inherent error in manual haematocrit determinations arises from trapping of plasma in the red cell column. This may account for 1 to 3% of the volume in microcapillary tube methods, with macrotube methods trapping more plasma [141]. In addition, it should be noted that abnormal red cells (such as sickle cells, microcytic cells, macrocytic cells, or spherocytes) might trap higher levels of plasma because of increased cellular rigidity, possibly accounting for up to 6% of the red cell volume [141]. Very high hematocrits, as in polycythemia, may also have excess plasma trapping. Manual haematocrit methods typically have a precision of about 2% (CV) [141].

Automated determination of haematocrit usually does not depend on centrifugation techniques, but instead measures or calculates haematocrit dependent on direct measurements of red cell number and red cell volume (haematocrit = red cell number

x red cell volume). The automated values closely parallel manually obtained haematocrit values, and the manual haematocrit method is used as the reference method for automated methods (with correction for the error induced by plasma trapping) [142]. It should be noted that errors of automated methods are more common in patients with polycythemia or abnormal plasma osmotic pressures. Manual methods of haematocrit determination may be preferable in these cases. The precision of most automated determinations of haematocrit is about 1% (CV) [143].

Red cell indices measurements

Cell counts remain the basis for many of the parameters used in evaluating the blood. These can be determined either manually or by automated haematology analysers. Whether they are performed by manual means or automated means, accuracy and precision of counts depends on proper dilution of samples and precise sample measurement. A precise aliquot must be taken and the cells evenly distributed within the sample. Because blood contains a large number of cells, sample dilution is required for accurate analysis. Clearly automated methods provide the best means for counting large number of cells and minimising statistical error.

Two major types of automated analysers are available, those that depend on changes in impedance to electrical flow and those that use differences in light scatter properties. The coefficient of variation for red cell counts for automated analyser is $\pm 1\%$ (cf manual $\pm 11\%$). Due to inherent imprecision of manual counts and the amount of technical time required most cell counting is now performed by automated or semiautomated instruments.

Aperture impedance counters

This type of analyser, which includes the Coulter (Hialeah, FL), the Sysmex (Baxter Diagnostics, Waukegan, IL), and some Cell-Dyn (Abbott Diagnostics, Santa Clara, CA) instruments, enumerates cells in a small aperture by measuring changes in electrical resistance as the cell passes through the orifice (Fig 11). A constant current passes between two platinum electrodes on either side of the orifice. The diluent that suspends the cells is more electrically conductive than are the cells. Hence, as each

cell passes through the orifice there is a momentary decrease in electrical conductance so that an electrical impulse is generated and recorded electronically. The drop in voltage is proportional to cell size, allowing average cell size to be determined simultaneously [144].

Instruments using aperture-impedance technology require even cell suspensions so that cells pass individually through the electrical current. Distortion of the electrical pulses may occur when the cells do not pass through the centre of the aperture or when more than one cell enters the aperture at a time. The Coulter type counters are probably the most widely used example of haematology analysers that use electrical impedance methods.

Optical Method Counters

The other method used by some haematology analysers depends on the light scatter properties of blood cells. In these systems, diluted blood passes through a flow cell detector placed in the path of a narrowly focused beam of light (usually a laser). When the blood cells pass through the counting chamber, they interrupt or alter the beam of light, thereby generating an electrical impulse that may be recorded.

6 *Measurement of plasma and blood volume*

i) Indicator dilution method

The plasma volume is commonly measured by dilution methods. A substance that is confined to the intravascular compartment such as I^{131} labelled albumin or radioactive indium labelled transferrin, [145,146], or Evan's Blue dye [147] is injected and the volume of distribution calculated from the degree of dilution of the injected substance over 15 – 30 minutes. Radiolabelled albumin is the most commonly used plasma label, but correction has to be made because the label is gradually removed from the circulation into the extravascular space [145,147] leading to errors of 10% or more in the plasma volume determinations. [145]

Total plasma volume may be useful in monitoring fluid and blood replacement. Total blood volume may be calculated from the sum of the total red cell volume and plasma volume measurements. The total blood volume comprises of red cell mass and plasma volume with an almost negligible contribution as a rule from the platelets and leucocytes. The ratio of the red cell mass to plasma volume differs in the venous system, the capillaries and the splenic blood pool.

In most cases the total number of erythrocytes is closely related to the red cell concentration of blood or the haematocrit. However in some cases the blood volume may not reflect the total red cell concentration including immediately following severe haemorrhage, severe dehydration and or overhydration. To accurately assess blood volume in these patients, plasma volume or red cell volume must be determined. The principle measurement of blood volume is dilution analysis. A small amount of readily identifiable radionuclide is injected IV either bound to cells or to a plasma protein and its dilution measured after complete mixing. Previously circulating Cr^{51} was used as a red cell label for measuring red cell volume. It is not ideal but is still used. Changes in plasma volume are achieved by alterations in distribution of water between intracellular and extracellular compartments across the capillary wall.

The venous haematocrit (PCV) is higher than the whole body haematocrit. The red cell mass does not fluctuate provided the circulation and erythropoiesis are in a

steady state. It is influenced by the age, sex, by compensation for increased oxygen needs and by other factors, which affect erythropoietin production. The plasma volume is more labile and affected by bed rest, exercise, food, posture, and ambient temperature. The total RBC count and PV do not necessarily reflect the total red cell volume. There is an exponential relationship between PCV and red cell volume but with wide dispersion.

ii) Indicator dilution methods – historical aspects

An indicator is a substance that can be easily identified when introduced into the circulation either instantaneously or by continuous infusion. Indicator dilution methods have been in use for many decades and have been useful in metabolic and circulatory studies. In 1824 Hering, professor of the Royal Veterinary School in Stuttgart first introduced an indicator dilution method for measuring blood velocity. [148] He injected potassium ferrocyanide into the jugular vein of 14 horses and sampled from other parts of the vascular system. Although the paper was titled “Experiments to measure velocity of blood circulation” he did not measure velocity but rather the time required for the indicator, injected instantaneously into one vein to be first detected in another vein by addition of ferric chloride to the blood sample to form Prussian Blue. 70 years after Hering, in 1897, Stewart from Cleveland suggested that the method could be used to measure blood flow and blood volume. He argued that the volume of solution necessary to dilute the indicator injected to the observed mean concentration of indicator output was exactly the volume of blood that had that had diluted the injectate over the time interval in which the indicator was recovered. [148] This formula was an important evolutionary step because eventually (30 yrs after the idea was first conceived) it paved the way for the use of indicator dilution curves to calculate blood flow and because it also suggested that the area under the indicator output concentration curve might have something to do with it.

The dominant figure in this field has been William F Hamilton with his many colleagues who used the technique with dye as the indicator. At first he measured appearance time. Then in 1928, 100 years after Hering he published a paper resurrecting Stewart’s formula. [148] He recognised that the calculation worked only

if the particles were calculated once. For more than two decades, beginning in 1928, Hamilton and his colleagues measured blood flow using cardiac output. They proposed that the first passage indicator concentration time curve could be recovered from observed curves that included recirculation by semi logarithmic extrapolation of the early downslope. Others followed with attempts to fit the complete first passage curve by various waveforms, such as by the sum of three exponential terms. Stephenson in 1948 thought of looking at indicator dilution curves as convolutions of the indicator input with a probability density function of traversal times through the system. [148] The fundamental notion is that there exists a probability density function of transit times (t) through the system. It has been proposed that the distribution of capillary critical opening pressures, which describes the recruitment of vascular pathways, may be important in shaping indicator dilution curve.

Hamilton was inspired to plot the indicator dilution curves semi logarithmically. He claimed that after an initial variation following the peak, the curve of first circulation fell as a single exponential line on the semilog plot. [149] Backextrapolation is an empirical method used to calculate central volume of distribution e.g. blood volume. It is based on the compartment model, which says that after an injection of a substance it is distributed instantaneously in the central volume with no time delay. The occurrence of recirculation is not taken into account. It was also found that the higher the rate of elimination of ICG, the higher the error of the backextrapolation method. [150,151]

Zierler first introduced the concept of dynamic volume. When sucrose, an extracellular tracer is injected along with a nondiffusible tracer (confined to the bloodstream), the difference between the extracellular and plasma volume is the interstitial fluid volume. [148]

iii) Measurement of plasma volume using indocyanine green

The measurements made of plasma and blood volume using indicator dilution technique on each subject are extensively discussed in the chapters on blood volume in the Results section. The tracer used for all experiments was Cardiogreen (ICG Green TM Sterile indocyanine green USP Fluka), a tricarboyanine dye, mol wt 775,

with an absorption peak at 805nm. The dye is nontoxic, confined to plasma, not subject to extravascular distribution, and not metabolised or degraded. Following injection, the dye is rapidly bound to plasma proteins. After equilibration, the dye decays fast in an exponential manner. It is exclusively taken up by the liver and has a plasma half-life 2-3 min (the time required for the initial concentration of the dye to be halved). [152] The elimination characteristics resemble the Michaelis-Menten kinetics [153].

7 Blood pressure measurements

i) Sphygmomanometry

Mercury column sphygmomanometry was used for manual reading by a trained clinician, taken in a seated position using the Phase 5 diastolic and a mean of two repeated measurements. The patient also recorded his/her own BP in the unit using an automated self-measurement Dinamap (Critikon)[9], [154] which downloaded the results directly into a software programme, blinded to the observer. Patients were well acquainted with the recording procedure, as it is a routine protocol in the unit prior to each dialysis. All casual measurements were taken in seated position with the arm resting and a cuff size suitable for the arm circumference attached to the nonfistula bearing upper arm.

ii) Ambulatory monitoring

For the purposes of this research, all patients studied were fitted with a TM-242 1, A&D Instruments Ltd. 48 hour ambulatory blood pressure monitor (ABPM). [155] This device measures BP by both the oscillometric and Korotkoff (via microphone) methods at pre-determined times which are fully programmable. The BP data is stored in memory and may be uploaded to a computer for analysis. In view of the fact that BP was also required during dialysis treatments, it was convenient to fit the monitor to the patient prior to the start of dialysis.

Tm2421 A&D Engineering, Milpitas, CA blood pressure monitor has been validated

and used for clinical and research purposes [10]. BP was measured using cuff size comparable to the seated BP measurements on the nonfistula bearing arm by a dual microphone system using Oscillometric (O) and Korotkoff (K) method programmed to record BP every 30 mins daytime and hourly at nighttime (2200-0700). Recorded data was retrieved, processed and reported using a computer software programme. 74.6% K readings and 95% O readings were successful. Accordingly the O method readings were used for the main analysis.

iii) Oscillometric BP

Patients were monitored frequently for blood pressure (Oscillometric) using the BPS08, (Fresenius Medical Care) during blood volume studies.

8 Bioimpedance Spectroscopy (BIS)

The hydration state of an organism can be defined by measuring the total body water relative to the lean body mass, defined as all nonlipid mass [156]. Traditionally this type of measurement required the use of research tools such as stable isotopes or underwater weights. Bioimpedance spectroscopy BIS emerged as a tool capable of accurate measurement of total body water. Fig 12 demonstrates possible disparities in fluid distribution between euvoletic normal subjects and in dialysis subjects in a clinical setting. More recently BIS has been shown to be capable of accurately measuring body water compartments.

i) Whole Body Multifrequency Bioimpedance

Bioimpedance is a noninvasive technique first applied by Thomasset in 1963. [157] In a typical application current is injected into the subject via a pair of electrodes placed on the ankle and wrist. An additional pair of electrodes monitors the resulting potential difference. The degree of conduction of current through the intracellular compartment is frequency dependent due to the presence of cell membrane that exhibits similar properties to those of an electrical capacitor. Since the potential

difference developed across the tissue also undergoes a phase shift with respect to those of the applied current due to the cell membrane, the overall measurement is also known as impedance signifying dependence on frequency. Application of a low frequency alternating current causes conduction almost exclusively through the extracellular tissues. At low frequency the cell membrane behaves as an insulator inhibiting the passage of current. In the high frequency range the cell membranes conduct and the current passes through the intra and extracellular spaces. (Fig 13)

HYDRA ECF-ICF (model 4200)

The instrument uses the latest in microprocessor, signal conversion, and digital processing technologies to produce a flexible, accurate and reliable instrument. The scope of measurement options enables the device to be used routinely for research.

Xitron's Bioimpedance spectroscopy (BIS) technology [158,159] has been validated on a number of populations by a variety of leading body composition research institutions dating back to 1992. [159,160] The studies have focussed on comparing predicted ECF-ICF volumes measured by wrist ankle to dilution and reference methods.

The current device has improved high frequency performance, thus providing improved prediction and accuracy and predictability. When quality data has been retrieved, a calculation of the ECF – ICF will take approximately 45 second. The ECF measurement itself takes 45 seconds.

Impedance measurement

The device measures the resistance (R) and reactance (X) and calculates the reciprocal impedance (Z) and phase angle (θ) at each measured frequency. The device provides a measure of complex impedance at different frequencies in a range according to a predefined mode. For ECF and ICF a spectrum of 50 frequencies spaced throughout a 5KHz to 1MHz. All frequencies are accurate to within a 0.05% of the selected frequency. A spectrum of 20 programmed frequencies spaced throughout a 5KHz to 200 KHz ranges. All measurements are performed using a

variable current of between 50uA and 700 uA dependent upon frequency output and load. The device has been classified as a Class I device. The resolution is 10 mls. [161]

Volume prediction accuracy

Validation research has shown that the correlation between predicted and dilution determined ECF range between 0.96 – 0.89. On HD pre and post dialysis correlation between the predicted and deuterium determined TBW was $r = 0.95$ and 0.92 respectively respectively [161].

The change in ECF compared to bromide dilution on critically ill patients was $R = 0.89$. On HD the predicted loss in TBW was reported to be within 0.8 kg of body weight loss. (161).

For continuous measurements, the relationship between ECF and body weight during dialysis was found to be $r = 0.83 - 0.99$, $0.76 - 0.99$ for ECF and ICF respectively. [162]

Volume prediction repeatability

The repeatability of the volume prediction is dependent on the resolution of the measurement modelling accuracy and the susceptibility of the electronic measurements to interference. It is influenced by electrode placement and biological variations such as volume of distribution, temperature and ion concentration change. Under ideal test conditions the repeatability is 50 mls when the measurements are performed repetitively.

Measurements during studies were made using standard tetrapolar lead arrangement with patients lying quietly for 5 mins. Impedance measurements were made using 50 logarithmic frequencies from 5 to 500 kHz. The software performs an iterative nonlinear curve fitting protocol for all spectra that conform to the Cole-Cole model of impedance spectra in living tissue. [161] Curve fitting yields electrical resistances (R) that correspond to extracellular water (ECF) R_e and total body water (TBW) R_{tbw} . The resistance of ICW R_i can be determined from R_e and R_{tbw} . By analysis of the impedance and phase shift at different frequencies, the resistance R_e (ECF)

and R_i (ICF) may be derived. Both these values depend on the volume of fluid in the respective tissue compartments. By combining anthropometric measurements of body segments and tissue resistivity constants determined from dilution studies, ECF and ICF can be studied. The availability of bioimpedance measurements have found widespread application in both dialysis and nutritional assessments. Although resistance can be converted to absolute volumes, this relies on derived equations in specific populations [161] or the application of complex physiologic principles of emulsion theory that may introduce unknown errors. Measurements were made on the dominant, nonfistula arm in case of dialysis patients. Patients on CAPD were evaluated post drainage of CAPD fluid. Each measurement was repeated in triplicate.

ii) THORACIC ELECTRICAL BIOIMPEDANCE

Over the past decade, electrical impedance cardiography has been developed as a noninvasive method to assess stroke volume and cardiac output. The technique determines the thoracic impedance changes resulting from pulsatory cardiac pump function. Because of the possibility of bedside measurement, it is suitable for monitoring during HD.

Impedance (inverse of conductivity) is a resistance to alternating current (AC). Bioimpedance is the impedance of body tissues. There is a direct relationship between bioimpedance of the segment and its pulsatile changes and the pulsatile blood flow through the segment. The total bioimpedance of the thorax (TFI =Thoracic fluid index) changes with the thoracic cross section and the thoracic fluid content. The majority of pulsatile impedance changes originate in the descending thoracic aorta. There are two sources for pulsatile impedance changes, volumetric caused by variation in blood volume in compliant arteries as a result of variation of arterial pressure and velocity caused by alignment of RBC from random (static blood at end of diastole) to preferentially aligned (with increasing velocity). Taken together the increase in blood volume and blood velocity during systolic upstroke contributes to changes in bioimpedance.

BOMED NCCOM3 - R7 Cardiodynamic monitor

(determination of haemodynamic variables)

The equipment is designed, configured according to BS 5274 standards, and is classified as Class II device and FDA registered. This is validated with the Biosym (microprocessor based, electrical bioimpedance device used to simulate cardiovascular parameters). Ejection fraction (EF) has been validated against MUGA scans. The accuracy of the haemodynamic measurement of cardiac index by any method including BoMed has an accuracy $\pm 10\%$. [163]

It measures impedance (resistance) to the high frequency measurement of AC current of the thorax. As more fluids are present within the thorax, it becomes more conductive. The device computes the blood flow and the thoracic fluid index data from the measurement of the thoracic bioelectrical bioimpedance and the ejection fraction (EF). The changes in the electrical conductivity of the thorax due to pulsatile flow of blood through the segment provide basis for TEB technology.

A variation in preload will result in a variation of stroke volume (SV). Increase in preload while the contractility, afterload and heart rate remain the same (in a hypothetical pump) will result in increase of stroke volume and hence an increase in pumping time.

Brief system description

The microcomputer board with its associated interface is the heart of the system. Two microprocessors operate at 5 and 10 MHz from the onboard oscillator. First TFI (base impedance value) is measured and stored by reading the A/D converter. The preejection period and ventricular ejection time (VET) and heart rate are measured by timing the pulses and the impedance measurement (dZ/dt) is taken and stored. The four measured parameters are then manipulated into formulae and the processor calculates the parameter for SV (stroke volume) as a function of height, weight, fluid index, ventricular ejection time and impedance changes. Cardiac output (CO) is a product of stroke volume and heart rate. Finally the other derivatives are calculated and stored in memory. Timing for the measurement of all the parameters begins with the incoming ECG impulses. Ecg QRS complexes trigger the processor into taking

the measurements and displaying them simultaneously. Good QRS complexes are very important in obtaining good measurement.

The resistance R_s is the equivalent resistance of the thorax and is actually varying in value as a function of blood flow. The constant current flow is 2.5 mA and causes a voltage drop across the resistance. As the value of R_s changes the respective voltage drop across the R_s varies and is detected by the Z amplifier. The ECG changes detected by the amplifier are used for internal timing of the measurements. The output of the Z amplifier is differentiated and along with the ECG signal is processed by the computer to derive the parameters. The total thoracic impedance is usually in the range of 10–48 Ohms; the small pulsatile changes, which are amplified and quantified, are the changes in the thoracic impedance due to blood flow. To assess reproducibility, Mehlsen et al tested 62 measurements of CO in a range 2.95 – 10.16 l/min with an SD 0.33 l/min (interobserver variation) and 0.12 l/min (intraobserver variation). [164, 165] It was concluded that impedance cardiography is reliable in measuring changes in CO and therefore suitable for repeated measurements in this study. Because aortic valvular pathology appeared to produce less reliable results, these patients with known aortic pathology or overt heart failure were excluded from the study while using this device. [166]

9 Data Acquisition and Processing

It was clear at the inception of the current research that a data set would need to be obtained measuring key haemodynamic parameters during fluid removal. In order to provide the most appropriate clinical interpretation of blood volume data it was essential that these variables could be acquired simultaneously. Consequently analytical methods developed to investigate the relationship between blood volume and fluid removal would then have a tenable link to known clinical indicators of fluid status.

A data acquisition system involving synchronised computers was constructed in order to allow simultaneous acquisition of these variables. Discrete asynchronous data were obtained at specific times during the treatment of interest, such as initiation and termination of an ultrafiltration pulse.

Each experiment required data acquisition from multiple monitoring devices at the same time integrated into a programme that was stored in a laptop computer. All these gadgets had to be in close proximity to the patient and the dialysis machine. Therefore a purpose built trolley was assembled containing the data acquisition systems, data concentrator, bioimpedance analyser and the cardiodynamic monitor. Location of the trolley at one side of the patient facilitated connection of the electrode leads to the patient, which are approximately 2 m in length in order to minimise the effects of stray capacitance. A purpose made umbilical cable was used to connect all of the data communication channels from the dialysis machine and associated sensors to the data concentrator. BP measurements were obtained from the nonfistula arm.

During each haemodialysis session, machine data was acquired and automatically downloaded to a specific program (Acqui, Fresenius) and stored in .dat files with real time data. The data was retrieved every 3 secs in a dat file format. This was subsequently converted to an Excel file for ease of analysis. An average file size for an individual study was 240 kb. The data downloaded included real time, count, haematocrit (%), Relative blood volume (%) blood flow rate (ml/min) Venous pressure and conductivity, Ultrafiltration rate and volume, blood pressure and temperature data. Appendix 1. Other physiological data including patient parameters were recorded in pre-printed sheets for each experiment.

Data processing and computational analysis

A large quantity of software was written in order to organise the data collected at sampling points, interpolate and filter data fields and implement a number of algorithms and produce file formats suitable for extracting specific information and further analysis.

Each patient study typically involved acquisition of a large quantity of data during a single treatment. Adequate methods by which data could be manipulated were a prerequisite for data analysis. This required a considerable quantity of dedicated software. The important functions performed by the dedicated software include

- Concentration and time synchronisation of data from different sources
- Filtering of data and generation of output files for graphical analysis and providing different cuts of the data for import into statistical packages.

- Implementation of special purpose algorithms for analysis of absolute blood volume data.
- Using specific data segments, automated curve fitting and derivation of fit constants using Table Curve 2D™ Software [167]

During analysis each .dat file was converted into an Excel format. Useful sections of the data could be selected out for specific analysis and transformed into .cut files for analysis. Specific analysis was performed using relevant portions of the data. Typical study setups involving multiple monitoring tools connected to a data acquisition system are depicted in the slides on Fig 14.

All Calculations and formulae and the mathematical software used for individual experiments have been referred to in the corresponding chapters. During blood volume studies, a specific algorithm was written to custom design an excel data sheet pre-programmed with blood volume equations to allow automatic derivation of volume measurements when the raw data was entered on the sheet for each patient. Such datasheets, which performed automatic calculations, were essential in saving time and minimising potential human error. Examples of such computer-generated sheets are depicted in Appendix 1. Statistical packages used were Sigmaplot, [168] SPSS and Systat software. [169, 170]

Clinical studies were performed according to specific protocols as outlined in the subsequent chapters. The North Herts Regional Ethics committee approved each study. Informed written consent was obtained from all subjects who participated in the research in all clinical studies.

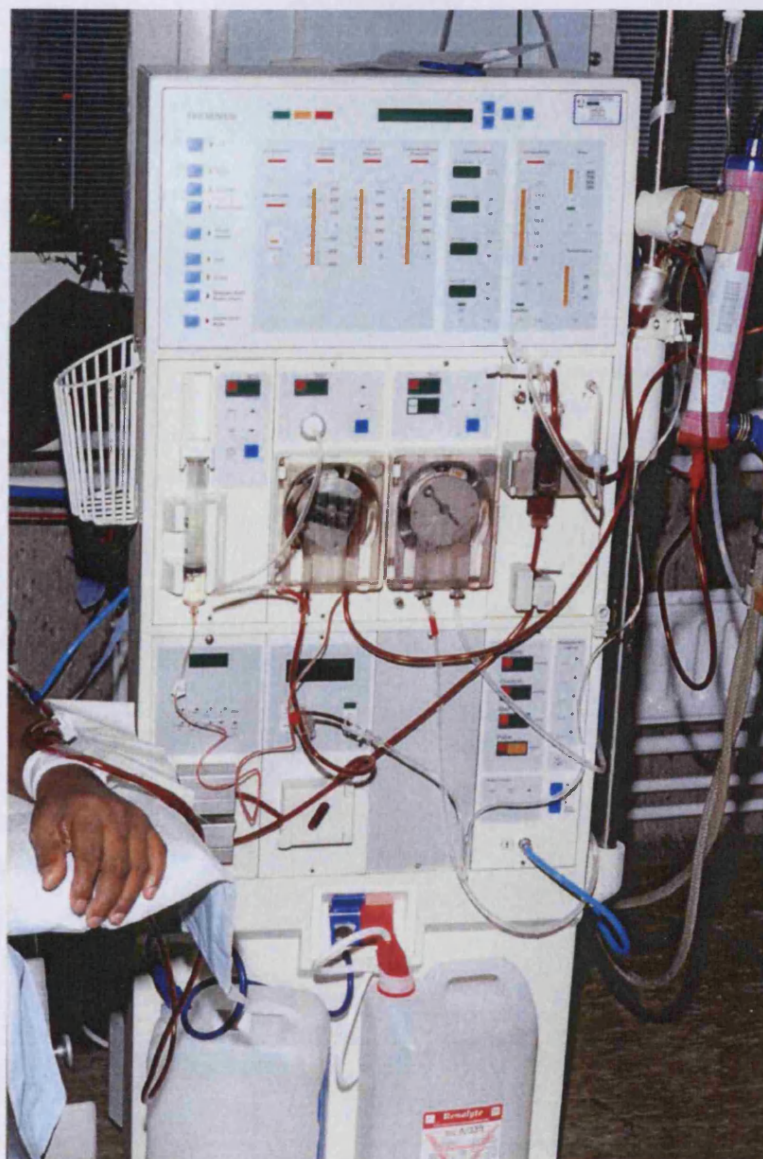


Figure 1 Fresenius Haemodialysis machine used for the clinical studies

Figure 2 A schematic diagram of the circuit and fluid pathway inside the HD apparatus

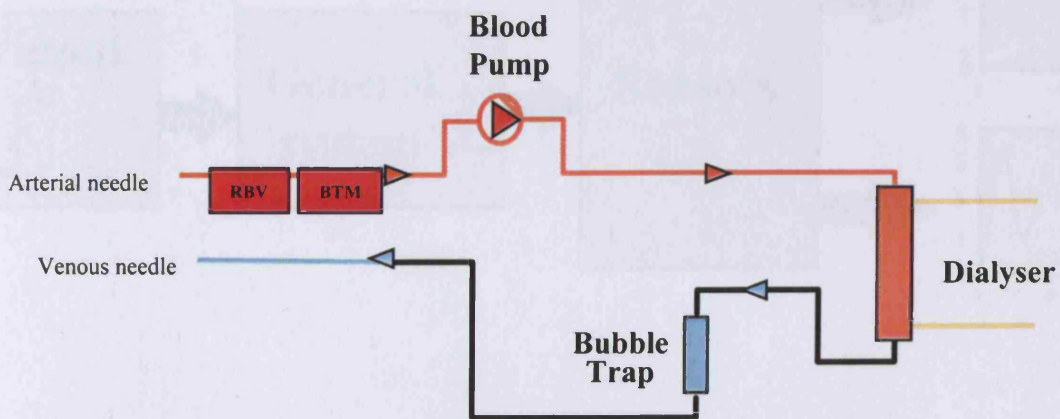
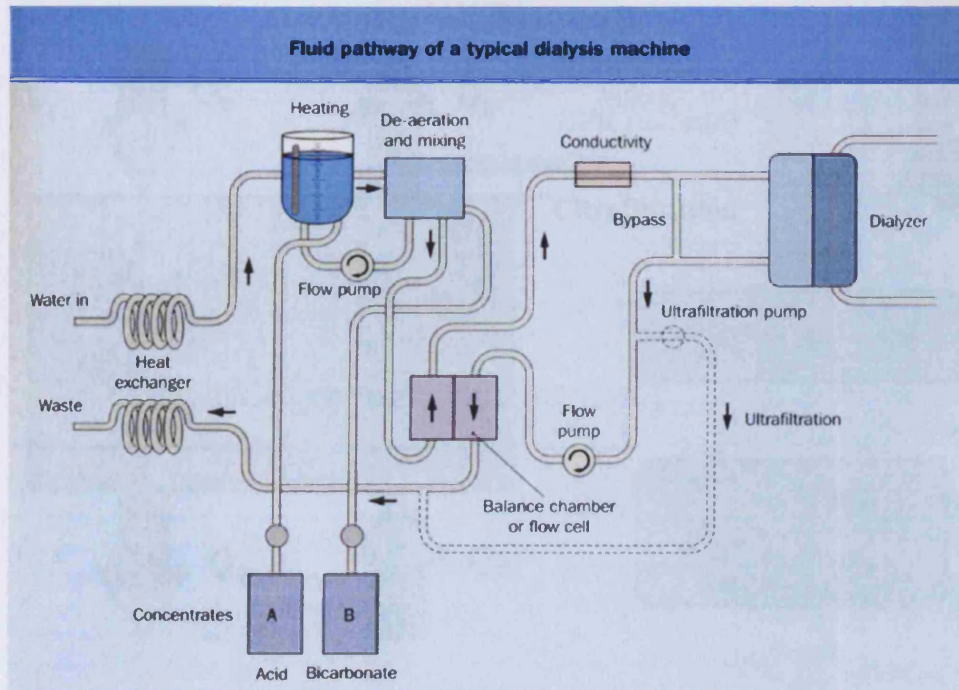


Figure 3 The Blood Circuit

Figure 4 The Ultrafiltration Coefficient (K_{uf})

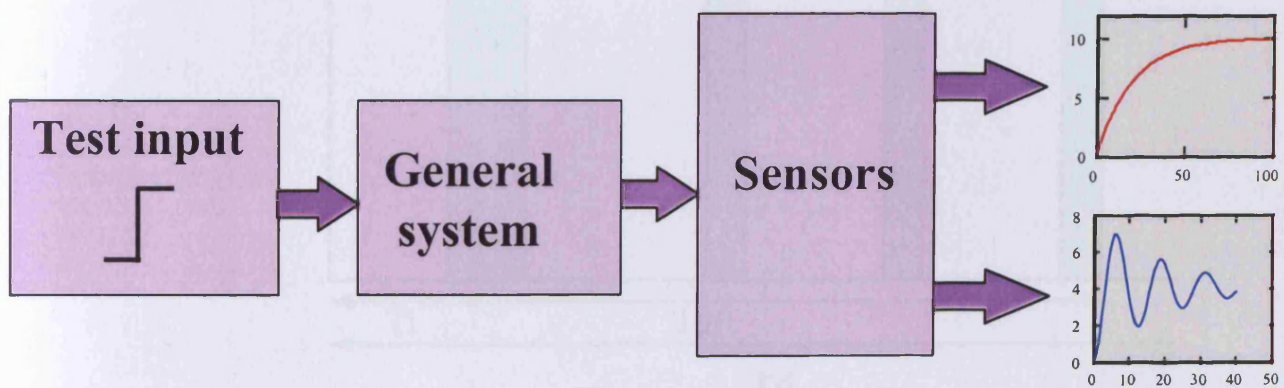
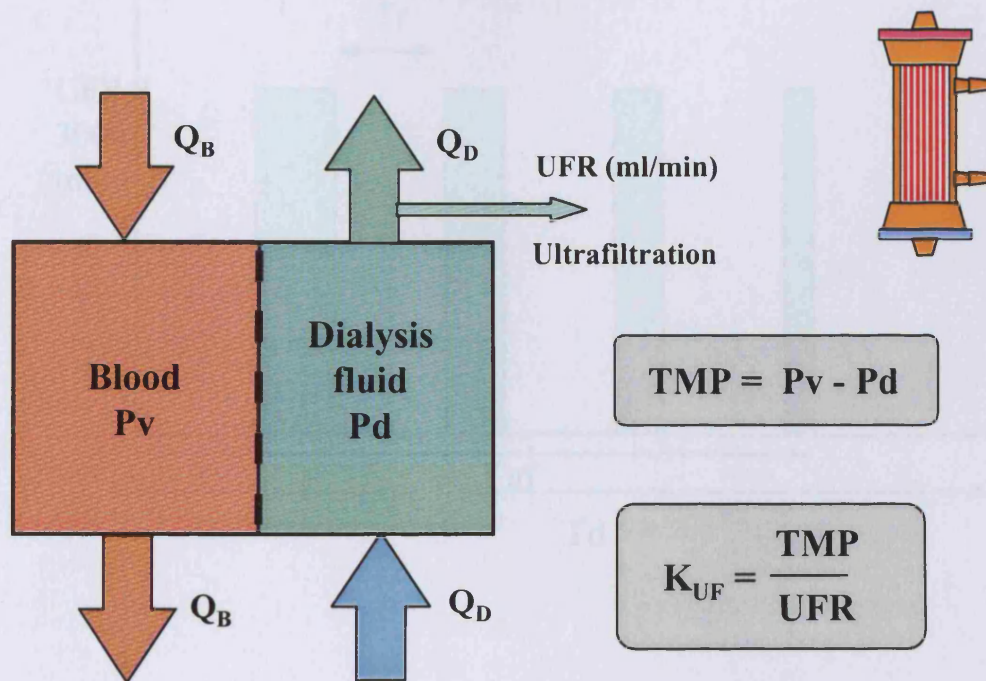


Figure 5 Principle of perturbation

Figure 6a Intermittent UF profile graded volume Protocol A

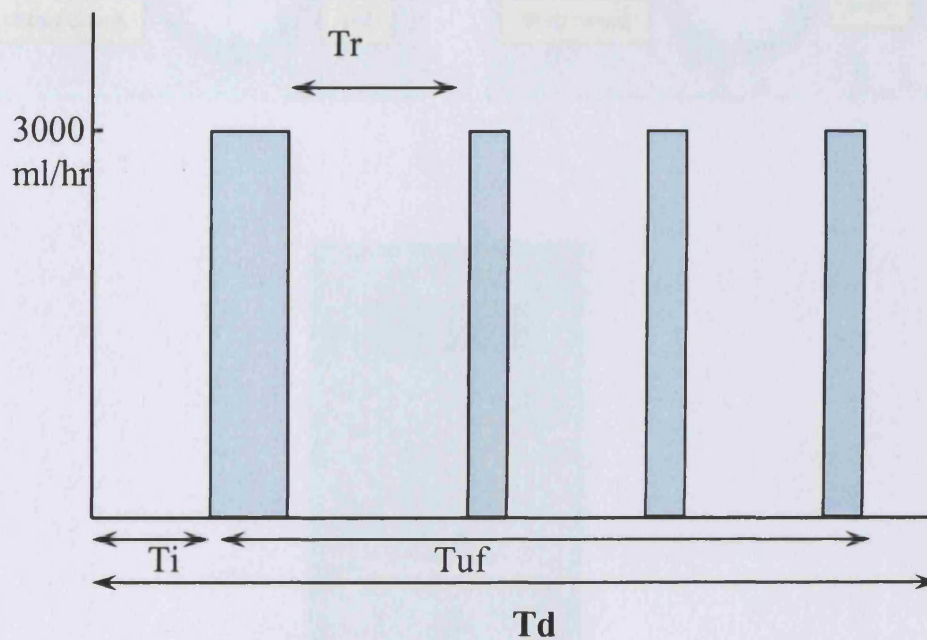
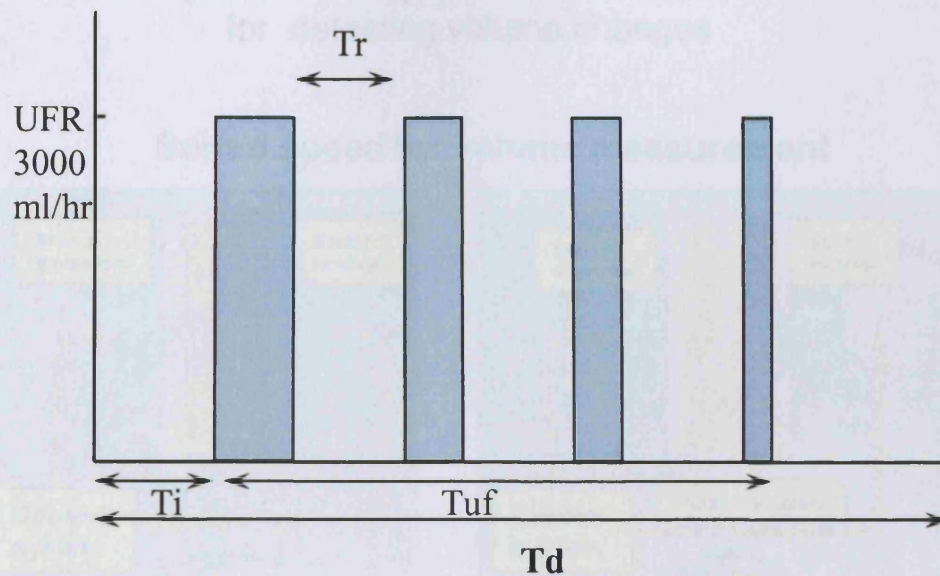


Figure 6b Intermittent UF profile Protocol B

T_d = dialysis + uf time; T_{uf} = total UF + refill time ;
 T_i = isovolemic period changes in blood volume during no UF
 T_r = time for refill period

Figure 7 Principle of ultrasonic device for detecting volume changes

Sound speed for volume measurement

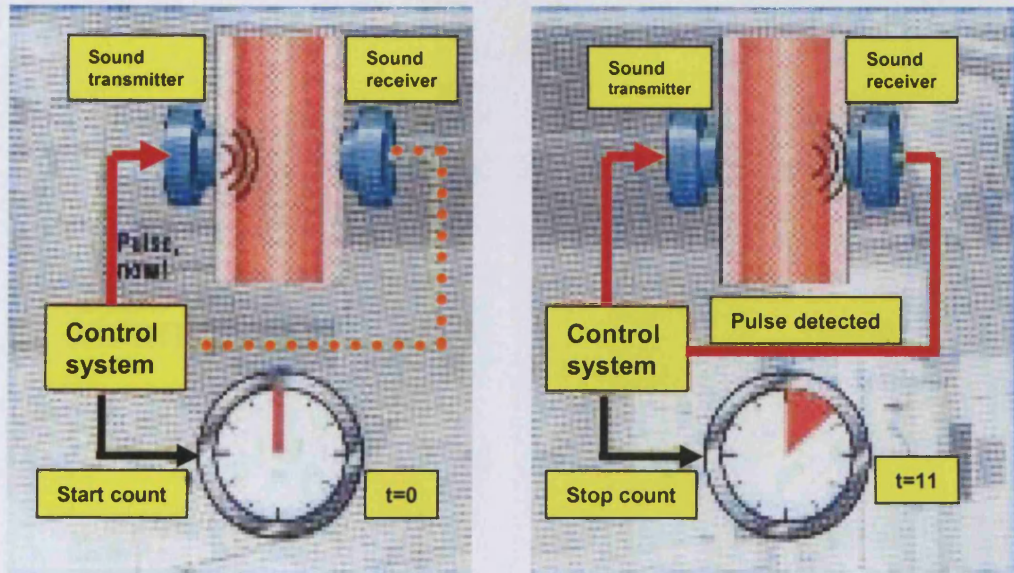
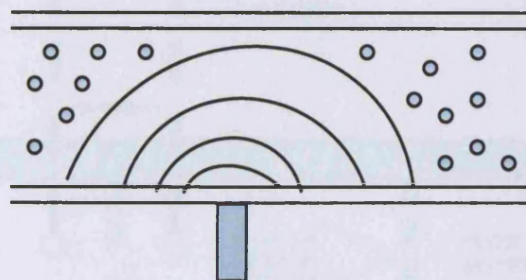
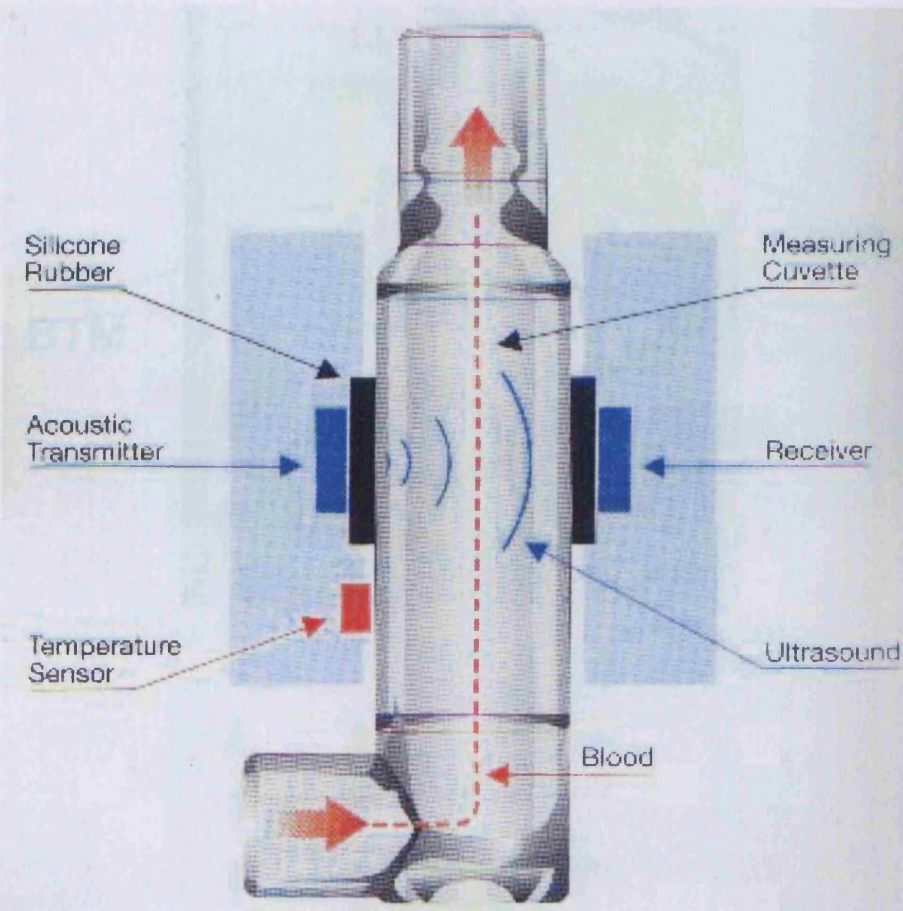


Figure 8 Fresenius blood volume monitor (BVM)

Figure 9 ULTRASONIC BVM SENSOR HEAD



crystal transducer
mechanism of action

Figure 11. Principle of capacitance measurement

Impedance type of measurement by capacitance

As the cells pass through the aperture they alter the capacitance between the electrodes, generating an electrical pulse.

The magnitude of the pulse is proportional to the cells volume.

Figure 10 Fresenius Blood temperature monitor(BTM) and BVM - Controls and connections

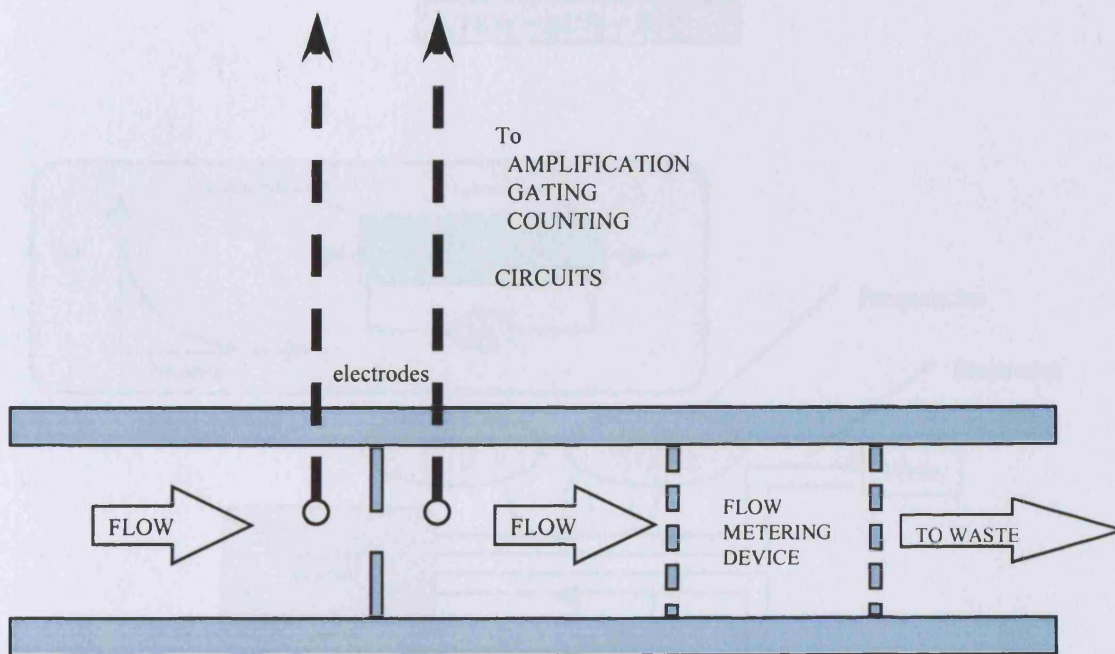


Figure 11 Principle of haematocrit measurement

Impedance type of haematology autoanalyser.

As the cells pass through the aperture, they alter the current flow between the electrodes, generating an electronic pulse.

The magnitude of the pulse is proportional to the cells volume.

Figure 12 Bioimpedance measurement of Bioratio (ECW/TBW) in overhydration and euolemia
 TBW = Total body water ;
 ECW = Extracellular water; ICW = Intracellular water

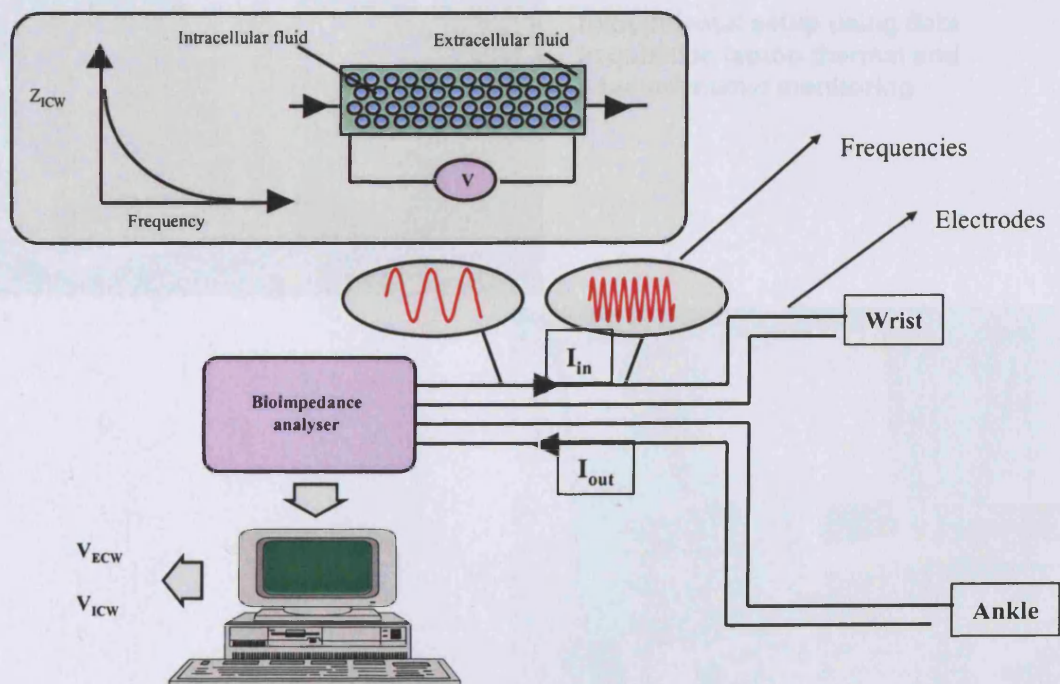
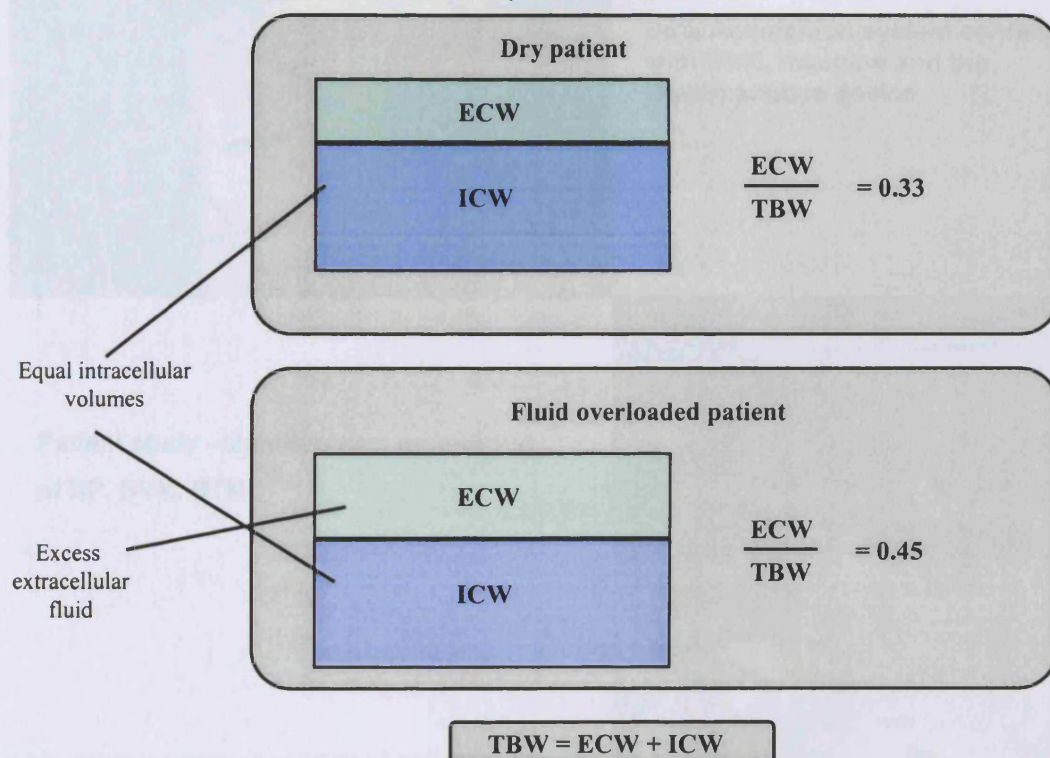
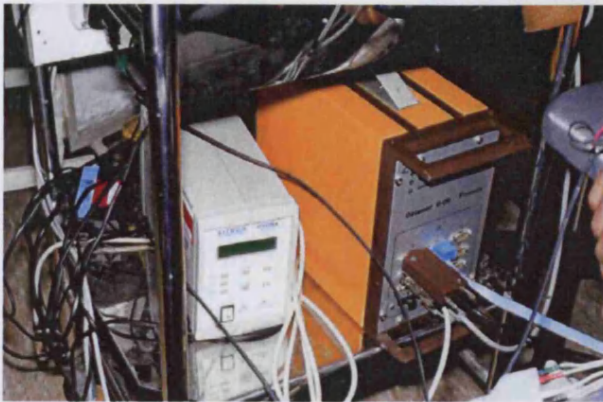
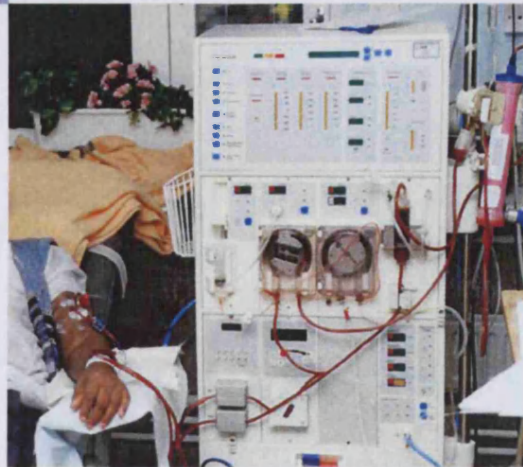


Figure 13 Measurement apparatus and principle of Bioimpedance analyser
 V_{ecw} = extracellular volume V_{icw} = intracellular volume Z_{icw} = impedance



Data Acquisition system connected with BVM, machine and the Bioimpedance device

Patient study –simultaneous monitoring of BP, BVM, BTM



Experimental setup using data acquisition laptop thermal and haemodynamic monitoring



Figure 14 Typical study setup using various monitoring tools

CLINICAL
EXPERIMENTAL STUDIES

Chapter 1

Blood pressure measurements in the interdialytic and intradialytic period and its relationship with changes in hydration status

Summary

Hypertension in chronic haemodialysis patients contributes significantly to morbidity and mortality. Treatment decisions are usually based on predialysis readings, which may not accurately reflect control during the interdialytic period. The relationship between BP and hydration status before and during dialysis with ultrafiltration is unclear.

In this experiment 40 randomly selected subjects on haemodialysis were studied by comparing BP readings by different methods at set times during the dialysis session with the 48-hr interdialytic ambulatory readings. Conventional sphygmomanometer, automated Dinamap and Tm 2421(A&D) ambulatory monitor were used for BP measurements. The relationship of BP with changing fluid status in the interdialytic and intradialytic period was explored. Results : Conventional sphygmomanometry and self measured automatic readings (Dinamap) were highly correlated (systolic $r=0.93$ $p<0.001$; diastolic $r=0.90$ $p<0.001$). Mean blood pressure on arrival [(preC₀) 158 mmHg systolic, 80mmHg diastolic and 106 mmHg mean) significantly overestimated the mean ambulatory reading during the 6 hrs prior to attendance [(preAm_{6hr}) systolic 147 ($p<0.01$), diastolic 75($p<0.01$), mean 99($p<0.01$)]. Fifteen patients (41%) demonstrated a marked difference ($>20/10$ mmHg) between the preC₀ and preAm_{6hr} (white coat effect) persisting in 7 pts (19%) after a period of rest 10 min predialysis (preC₁₀) and present even in self recorded Dinamap readings. There was a significant negative relationship between the systolic rise and the number of months on dialysis ($p<0.05$). Mean ambulatory BP on interdialytic day 2 was significantly greater than on day 1 whereas the awake-sleep differences were less on day 2 than day 1, both perhaps reflecting differences in volume status. The 20 min post dialysis measurement PoC₂₀ for systolic, diastolic and mean unlike predialysis (preC₀ and preC₁₀), onset (onC) and end of dialysis readings (enC) did not differ significantly from 48 hr interdialytic means.

Introduction

Hypertension in chronic haemodialysis (HD) patients contributes significantly to their morbidity and mortality. Epidemiological studies have consistently shown that, although treated, blood pressure (BP) frequently remains inadequately controlled in a high proportion of HD patients [28,171]. This is complicated by the high morbidity of both over and undertreatment [172,173] and by factors peculiar to the HD situation itself.

Although multifactorial, salt and water excess remain the predominant contributory factor to dialysis hypertension. Adequate removal of salt and water by ultrafiltration therefore can control a significant proportion of elevated BP in the HD population. Vasoactive factors seem to operate in relation to a degree of fluid excess in hypertensive individuals. Achieving normotension remains a clinical end point in the process of dry weight attainment in many subjects on haemodialysis.

It is however difficult to define hypertension in dialysis. Which blood pressure reading should be taken to signify hypertension is more pertinent in dialysed individuals than in the general population because of their fluctuating fluid status and other factors associated with the dialysis session. In the general hypertensive population it is known that the use of single readings as a reliable indicator of the overall BP control is fraught with difficulty because of transient and persistent elevations of pressure in the clinical setting [174]. The variability of casual readings in relation to the dialysis cycle confound management decisions and pose a dilemma with regard to the optimum timing and method of measurement of BP in this setting. Treatment decisions are mostly based on predialysis readings. However the relevance of these readings has been questioned and studies have shown their tendency to overestimate true BP [175]. Epidemiological studies also suggest ambulatory BP control may be a better predictor of target organ damage [176]. 40 randomly selected patients undergoing haemodialysis were investigated without any alteration in medication or dialysis schedule with the aim of comparing methods of measurement and comparing casual readings during the dialysis session with 48 hr interdialytic ambulatory readings. An attempt was made to explore the relationship between changing hydration and overall BP control in these subjects.

Patients

Forty randomly selected stable chronic stable HD patients attending the dialysis unit were studied. The patient characteristics of the study group are shown in Table 1. All patients received high flux dialysis 3 times per week using bicarbonate buffer. Dialysis was prescribed according to a urea kinetic model [112] with mean dialysis duration 160 ± 43 mins. They reached their dry weight without hypotension and severe cramps and had acceptable interdialytic weight gains. Sixteen patients dialysed in the morning (1030-1200 hrs) and 24 pts in the afternoon (1230-1600 hrs). Nine (22%) were on no antihypertensive medications, 55% were on mono or dual therapy. Drugs used were ACE inhibitors (22 patients), calcium antagonists (14 patients) and beta blockers (3 patients). Antihypertensive medications were not withheld on the day of dialysis and usually taken in the morning. Patients excluded were a) those on twice weekly HD b) those in atrial fibrillation c) those who had undergone hospital admission within the previous month and d) those in whom antihypertensive agents had been altered within the previous 2 weeks e) those on early morning or evening dialysis shifts. Four patients had taken off their ambulatory monitor a few hours before arriving in the unit and the results were not used in analysing the white coat effect.

Methods

Blood pressure measurements

- (1) Mercury column sphygmomanometry was used for manual reading by a trained clinician using the Phase 5 diastolic and a mean of two repeated measurements.
- (2) The patient also recorded his/her own BP in the unit using an automated self-measurement Dinamap (Critikon)[177], which downloaded the results directly into a software programme, blinded to the observer. Patients were well acquainted with the recording procedure, as it is a routine protocol in the unit prior to each dialysis. All measurements were made in the sitting position. A cuff size suitable for the arm circumference was selected.
- (3) Ambulatory monitoring was carried out using the Tm2421 A&D Engineering,

Milpitas, CA blood pressure monitor. This has been validated and used for clinical and research purposes [178]. BP was measured using cuff size comparable to the seated BP measurements on the nonfistula bearing arm by a dual microphone system using Oscillometric (O) and Korotkoff (K) method programmed to record BP every 30 mins daytime and hourly at nighttime (2200-0700). Recorded data was retrieved, processed and reported using a computer software programme. 74.6% K readings and 95% O readings were successful. Accordingly the O method readings were used for the main analysis.

The width of bladder 40% arm circumference and length of bladder 80 % arm circumference is considered ideal. The cuff sizes used were according to the British Hypertension Society recommendations. The standard cuff range for adults used were comparable with respect to different measurement methods used in this study and measured 23-33cm (Dinamap and Sphygmomanometer) and 21-32 cm for ABPM. For larger individuals the cuff ranges were 31-40cm (Dinamap and Sphygmomanometer) and 30-38cm for ABPM.

Definitions used

Conceptual average BP Mean predialysis BP from last 10 visits for HD as recorded

on the database.

Systolic load % of all ambulatory readings >140 mmHg [179]

Diastolic load % of all ambulatory readings >90 mmHg [179]

Dippers > 10% fall in the mean pressure during the night (2200-0700)

compared to daytime readings [180]

Awake-sleep difference Difference of mean day and night time BP in the 48-hr period.

White coat effect Rise in BP of >20/10 mmHg in the reading on attendance to the unit above the daytime ambulatory BP during the 6 hrs prior to attending the unit.

Best representative casual reading defined as that showing the minimal difference from the mean during the interdialytic period [179]

Average BP (AvC)	Mean of pre (PreC ₁₀) and post (PoC ₂₀) readings.
Hypertension (casual reading)	Systolic>150 or diastolic >90 mmHg
Hypertension on 48hr abpm	Systolic>135 or diastolic >85 mmHg [181]
Daytime hypertension on abpm	Systolic>140 or diastolic >90 mmHg [181]

Protocol

BP on arrival for dialysis (PreC₀)was checked by the patients themselves using the Dinamap and then by a clinician using sphygmomanometer. The patient then rested in a quiet room for 10 minutes after which BP measurement was repeated (PreC₁₀) in a similar manner (by the patient using a Dinamap and by the clinician using sphygmomanometer). An ambulatory monitor was then attached to the patient with an initial reading on the monitor checked to ensure reading coincided within 5mm of the sphygmomanometer reading [182]. During dialysis BP was recorded at the onset (onC), end (enC) and post dialysis prior to leaving the unit (PoC₂₀) 15 –25 mins from end of dialysis (mean time 20 mins from end of dialysis). On arrival for the next dialysis the monitor was removed and BP checked as before by the patient using Dinamap and the clinician using the sphygmomanometer before and after a period of 10-min rest. All casual measurements were taken in seated position with the arm resting and the cuff attached to the nonfistula bearing upper arm.

Statistics

The statistical tests used were the Students' t-test (paired, one sample or independent sample) for differences between means and Pearson correlation coefficient. ANOVA and stepwise multiple regression analysis were used to explore relationships between variables. All analyses were done using the SSPS statistical package. Bland-Altman analysis was used for comparison of methods [183].

Results

Ambulatory BP readings

The average systolic, diastolic and mean BP of all patients over a 48-hr period was 140, 71 and 94 mmHg respectively. The mean daytime and night time values for systolic, diastolic and mean BP were significantly different (Table 2). Only 8 patients (20%) were dippers. This abnormal nocturnal pattern of BP has been recognised in other studies and is typical of the hypertensive HD population [184]. Overall systolic and diastolic load was 42 % and 15.7% respectively.

Comparison of methods

Systolic and diastolic pressure measured by conventional sphygmomanometry showed a highly significant correlation with the automatic self measured counterparts (Fig1) Bland-Altman analysis showed an acceptable mean difference of -1.8mm(systolic) and 0.6mm(diastolic) with limits of agreement (2 SE mean difference) of +6 to -5 (diastolic) and 8 to -11 (systolic) mmHg from the mean of the two methods (Fig 2).

Comparison of casual readings during dialysis cycle with ambulatory readings [Tables4& 5]

Predialysis readings significantly overestimated the ambulatory averages. $PreC_{10}$ though significantly lower than $PreC_0$ ($p < 0.01$), was still an overestimate of the ambulatory mean readings. While predialysis reading overestimated the ambulatory mean, measurement at the end of dialysis (enC) significantly underestimated it. The 20-min post dialysis readings (PoC_{20}) slightly underestimated (-4 mm mean systolic and -1.7mm mean diastolic) but were not significantly different from ambulatory means. PoC_{20} provided the best single reading representation of the average interdialytic BP. A reliable estimate could also be obtained by averaging pre and post measurements (AvC). The variability of these blood pressures is depicted in the Fig 3 & Fig 4.

Assessing white coat effect

Blood pressure on arrival to the dialysis unit significantly overestimated the ambulatory averages for 6 hours prior to arrival by $>20/10$ mmHg (white coat effect) in 15 of 36 patients who completed 48 hr monitoring (41%). The mean rise in these patients was 25mmHg systolic and 13 mm Hg diastolic ($p<0.01$). This effect persisted in 7 patients (19%) following a repeat measurement after 10 mins rest (25.3 mm Hg systolic and 16 mm Hg diastolic; $p<0.01$). The white coat effect was observed consistently in both sphygmomanometer and Dinamap readings. In those demonstrating the effect the mean difference between clinician measured and self measured readings were 2mmHg systolic and 0.5 mmHg diastolic ($p=ns$). The white coat effect was calculated by comparing casual readings on arrival with the daytime average of readings taken in the 6 hrs prior to arrival for dialysis because during this period patients were at their wettest and BP differences were less likely to reflect fluid gains. The incidence of the white coat effect was greater if the entire 48-hr interval (23 patients) or entire 48 hr mean daytime readings (18 patients) were used as comparators with the arrival BP. The reduction in mean (18 vs 10 mmHg) and systolic BP (38 vs 14 mmHg) during dialysis in the group with white coat effect was significantly higher than in the patients not demonstrating the effect showing that the effect was not sustained during or after dialysis. Patients who demonstrated the white coat effect had been on dialysis for fewer months and showed a higher awake-sleep difference (10.5 vs 3.2 systolic; 5.8 vs 3.5 diastolic) in the interdialytic period ($p<0.05$ for systolic) when compared with those not demonstrating the effect. Daytime systolic readings (139 vs 144 mmHg) and overall pressure load in this group were also slightly lower than the rest while the mean weekly erythropoietin was slightly higher (Table3). The latter differences were not significant. There was no significant difference in those showing the white coat effect and others with respect to gender, age, drug treatment, interdialytic weight gain, residual renal function, KT/V, timing of the dialysis session or the proportion of dippers (Table3). The observations were similar in those who had persistent white coat effect after a period of rest.

Stepwise multiple regression analysis in those with white coat effect, using systolic rise as the dependent variable showed a negative relationship with the number of months on dialysis ($p < 0.05$, $r = -0.62$). No significant predictive effect was observed for other independent variables including age, sex, number of antihypertensive drugs, 48hr average BP, daytime average BP, duration of dialysis, pulse rate or awake-sleep difference. Fig 5 demonstrates case studies of three patients depicting ABPM profile in the interdialytic period.

Relationship to interdialytic fluid gains

A significant relationship of weight gain or ultrafiltration volume with casual BP on dialysis, white coat effect or nocturnal dipping could not be demonstrated except for a weak but significant correlation between the BP at the end of dialysis (EnC) and ultrafiltration volume ($p < 0.05$; $r = -0.40$). During the interdialytic period however, average ambulatory BP on Day 2 was higher than on Day 1 (systolic 147 vs 139mm $p < 0.05$; diastolic 74 vs 71mm $p = \text{ns}$). The awake-sleep difference was significantly reduced on Day 2 compared with Day 1 (1.6 vs 7.5mm Hg systolic; $p < 0.05$).

Discussion

The measurement of BP for clinical evaluation of the HD patient may be subject to three types of error. First is the error due to the measurement procedure itself. We confirmed that same arm BP measurements by Dinamap and the clinician are comparable though not necessarily identical [185]. The agreement between mercury column and Dinamap determinations were within 10mm Hg in 90% systolic and 98% of diastolic readings. The error between two devices may be due to a) consecutive rather than simultaneous readings or b) systematic differences which can be corrected for by adjusting for the average difference (negligible in this study) between the devices. The overall level of agreement is reassuring and suggests that either may be used with confidence in the clinical setting.

The second type of error arises from the spurious elevation of BP in the clinical setting often attributed to an emotional reaction to the involvement of a clinician. The “white coat effect” is defined as transient rise in BP that occurs in the clinical

setting [186]. We observed this phenomenon in almost 40% of arrival pressures, which improved to 19% after a period of rest. The effect did not persist throughout the dialysis session (Fig 6). The timing of the dialysis session did not influence the presence of WCE. There seemed to be no noticeable difference when the physician took the BP. The involvement of the physician does not seem to be a determining factor in the white coat effect. This study confirms that the white coat effect persists even in patients on antihypertensive therapy [187]. The negative relationship with duration on dialysis could be due to habituation to clinic measurements with time or due to the longer duration of antihypertensive therapy.

There is little data on the white coat phenomenon in the dialysis population, though in a study on 13 normotensive HD patients [188] large differences were noted between the predialysis readings and interval pressures just before dialysis. The timing of the predialysis blood pressure measurement is therefore crucial. The initial arousal response can be minimised using readings after a rest period. The mechanisms and prognostic significance of the white coat effect are not fully understood [182]. The response occurs in about 20% of the general hypertensive population [189], is more common in females and blacks and usually disappears on repeated measurements. In the nondialysis population it is associated with elevated plasma level of various hormones including catecholamines, cortisol, vasopressin, endorphins and a primary role of the sympathetic system has been suggested [190]. The white coat phenomenon may be more common in renal patients than in the general hypertensive population [191] perhaps due to an exaggerated sympathetic response conditioned by uremia. It is noticeable (Fig5) that in the group not demonstrating the white coat effect WCE (-) there appeared to be a gradual rise in BP between dialyses whereas in the group demonstrating the effect WCE (+) BP seemed stable in the interdialytic period before an abrupt predialysis rise. Perhaps there is an increased sensitivity to fluid accumulation in the WCE (-) group, which may be determined by a sustained sympathetic outflow, whereas the sympathetic surge in the WCE (+) group is more transient. The environment of a busy haemodialysis unit may have a significant influence on the predialysis surge, as may the pressures of travelling to the unit and the fear of needles. Because of the unpredictability of the effect, we recommend that this be carefully considered when assessing resistant predialysis hypertension.

The third error arises from the variability of BP during the dialysis cycle. The large swings in pressure during the dialysis procedure are not fully understood. The overdiagnosis of hypertension using predialysis readings has been observed in many studies [175]. The practice of withholding antihypertensive medication predialysis has been implicated [28] but was not a factor in this study. Even after elimination of the initial arousal phenomenon, predialysis BP poorly reflected interval pressures [175]. The changes in BP during dialysis could reflect volume removal, removal of vasoactive factors, and alterations in arterial compliance and sympathetic activity caused by removal of uremic toxins. Despite poor correlation of blood pressures on dialysis with weight gains, the differences in Day 2 and Day 1 average daytime, nighttime BP and awake-sleep differences suggest some influence of fluid accumulation on diurnal fluctuations of 48 hr BP between dialyses.

The 20-min post dialytic BP (PoC₂₀) appears to be best approximation of the ambulatory interdialytic values in the stable chronic dialysis population. Similar findings have been reported by Kooman et al [175] while others report unacceptable variability in the predialytic reading [182]. There is significant underestimation of interdialytic means if BP is recorded immediately after dialysis. In our patients the fall in arterial pressure during dialysis recovered rather than dipped post dialysis though Battle et al observed a decline in BP in the hours immediately after dialysis [192]. The variation may relate to differences in baseline hydration. The recovery of the 20 mins post dialysis reading may be related to compensatory vascular refilling (fluid rebound) which is maximal in the first half hour post ultrafiltration [193]. Allowing this period of equilibration is essential to obtain reliable post dialysis measurements.

Those not on antihypertensive drugs had significantly lower interdialytic weight gains ($p < 0.05$), higher residual renal function and were on less Erythropoietin. They did not however differ significantly with respect to overall BP control, WCE or BP fluctuations during dialysis. They numbers are however relatively small to be conclusive.

In conclusion the timing of casual predialysis and postdialysis BP readings is crucial. Both can be biased by the dialysis procedure itself (white coat effect in the former

and fluid rebound in the latter). The traditional diagnosis of hypertension based solely on predialysis clinic readings can lead to gross overestimation attributable to a white coat effect. This is an important cause of blood pressure variability and is significant even in self-recorded blood pressure measurements. The best single approximation of interdialytic BP is the 20-min postdialytic reading. Poor control or sustained hypertension with high clinic BP, despite multiple drug treatment should be assessed by ambulatory monitoring to eliminate overshooting reactions, provide supplemental information about pressure load and variability and avoid inappropriate treatment. Prospective endpoint studies are required to authenticate the relationship between ambulatory BP values and measurements in the dialysis units and the use of both in predicting cardiovascular morbidity and mortality in HD patients.

Table 1. Patient characteristics

	Mean	SD	Range
Age (yrs)	61.5	(14.8)	21- 81
Months on HD	27	(18.7)	6 - 78
IDWG (kg)	1.34	(0.72)	0.4 - 3.7
Drugs	1.5	(1.0)	0 - 4
Epo (IU/week)	6000	(4600)	0 -20000
KRU (ml/min)	1.7	(1.73)	0 – 4.8
Kt/V	1.23	(0.19)	0.9 – 1.8
Ultrafiltration (l/hr)	0.59	(0.26)	0.19 – 1.2
Sex m:f	35:5		
Diabetic	10		

Table 1 Patient Characteristics at the time of enrolment into the study.

KT/V = mean of delivered KT/V measurements performed within the 4 months prior to the study.

KRU = residual renal function as urea clearances (ml/min).

Drugs = No of antihypertensive medications. Epo = Erythropoietin dosage

IDWG = interdialytic weight gain (kg) SD = standard deviation.

Table 2. Interdialytic Blood Pressure Monitoring

	48 hr	Day	Night	D-N diff
Systolic	140(21)	142(21.3)	135.7 (24)	p<0.001
Diastolic	71.7(11)	72 (10.1)	68.2(12)	p<0.001
MBP	95 (14.5)	95.3 (13)	91(15.4)	p<0.001
Systolic load	42.3% (31)	Diastolic load 15.7% (16)		

Table 2 Average BP and pressure loads during the 48 hr interdialytic monitoring.

Figures in parentheses indicate the standard deviation of the mean.

MBP = mean blood pressure D-N diff = day night difference.

Table 3 . Comparison of groups with or without white coat effect (WCE) on arrival predialysis measurement (PreC₀).

	WCE absent		WCE present	
Number	21 patients		15 patients	
Sex (F:M)	2:19		3:12	
Diabetic vs nondiabetics	6:15 (28%)		4:11 (27%)	
	<u>Mean</u>	<u>SD</u>	<u>Mean</u>	<u>SD</u>
Age (yrs)	65	14	58	14
Months on Haemodialysis *	29	19	17	11
No. of anti-hypertensive drugs	1.5	1	1.6	1
Weekly Epo Dose (units)	5433	3166	7167	3824
Inter-dialytic Weight Gain (kg)	1.27	.58	1.35	.84
KT/V	1.21	.20	1.24	.24
Conceptual average systolic BP *	150	11	176	12
Conceptual average diastolic BP *	73	10	90	14
Average 48 Hour Diastolic BP	71	11	72	11
Average 48 Hour Mean BP	95	13	94	11
Average 48 Hours Systolic BP	141	24	137	18
Average Daytime Diastolic BP	72	10	74	12
Average Daytime Mean BP	95	15	94	10
Average Daytime Systolic BP	139	25	144	18
Average Night time Diastolic BP	68	12	70	16
Average Night time Mean BP	92	17	90	14
Average Night time Systolic BP	137	27	133	20
Diastolic load (%)	14	18	12	13
Systolic load (%)	43	32	41	29
Pulse (per min)	72	10.7	74	7.5
48hrAwake_sleep diastolic difference	3.5	4.3	5.8	7.1
48hrAwake_sleep systolic difference*	3.2	5.5	10.5	4.5

(*) indicate significance of p<0.05. WCE = white coat effect.

Abbreviations = refer to Table 1.

Table 4. Blood Pressure measurements during dialysis cycle compared with interdialytic pressures

	Systolic	Diastolic	MBP
48 hr average	140 (21.5)	71 (11)	94 (13)
Conceptual Average	157 (22)	78 (10)	104 (12)
PreC ₀	158 (20)	80 (11)	106 (12)
PreC ₁₀	150 (21)	74 (10)	99 (12)
OnC	146 (23)	76 (16)	99 (15)
EnC	124 (31)	70 (13)*	88 (16)
PoC ₂₀	136 (25)*	70 (11)*	92 (14)*
AvC	144 (21)	74 (10)	97 (12)

Table 4 Blood Pressure measurements during dialysis cycle compared with interdialytic pressures.

* p = ns (no sig diff from the 48 hr mean). Students t- test paired with 48 hr readings for systolic, diastolic and mean. Values rounded to the nearest integer.

PreC₀ =Predialysis BP on arrival, PreC₁₀=10 min predialysis BP , OnC=onset of dialysis BP, EnC=BP end of dialysis, PoC₂₀=20 min postdialysis BP, AvC=Average of pre and postdialysis BP
MBP = mean blood pressure.

Table 5. Mean differences of BP during dialysis from the average 48 hr ambulatory pressures

	Systolic	Diastolic	MBP
PreC ₀	18.3 (2.5)	7.0 (1.3)	11.5 (1.6)
PreC ₁₀	9.2 (2.3)	2.7 (1.1)	5.1 (1.5)
OnC	5.1 (3.0)	4.5 (1.8)	5.3 (1.7)
EnC	-14.1 (2.3)	-3.2* (1.5)	-6.6 (1.6)
PoC ₂₀	-4.0* (2.3)	-1.7* (1.4)	-1.8*(1.6)
AvC	4.3 (1.9)	1.9* (1.1)	3.0 (1.3)

Table 5 Mean differences of BP during dialysis from the average 48 hr ambulatory pressures.

* p = ns (no significant diff from the 48 hr mean)Figures in parentheses indicate the sd of the mean.

MBP = mean blood pressure. Key to abbreviations in first column refer to legend Table 4.

Figure 1 Correlation of blood pressure measurements between Sphygmomanometer and Dinamap for systolic (1a) and diastolic (1b) on arrival to the unit.

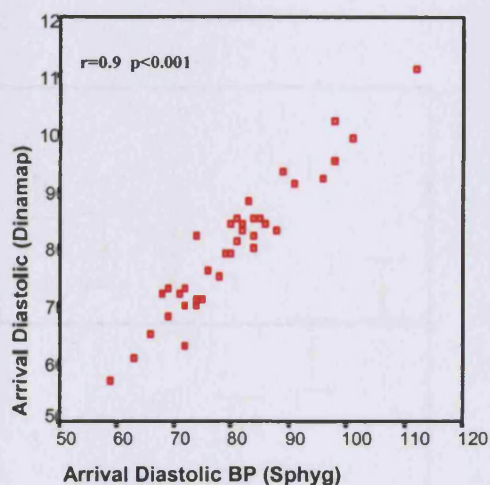


Fig1b Diastolic

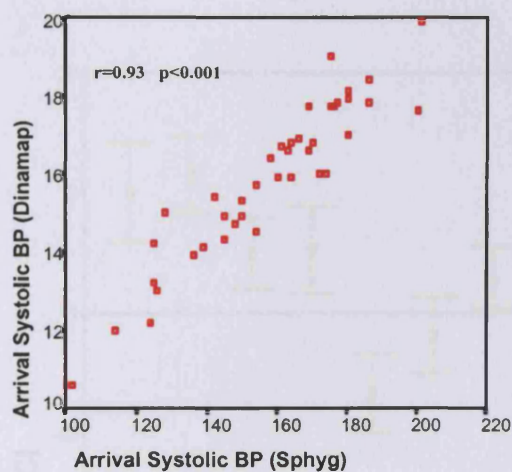


Fig 1a Systolic

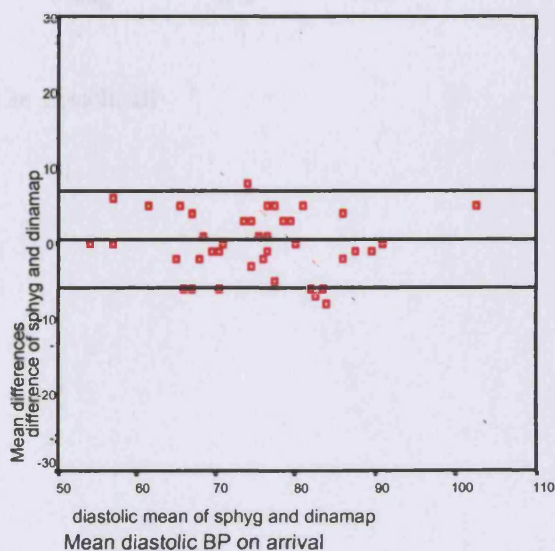


Fig2b Diastolic

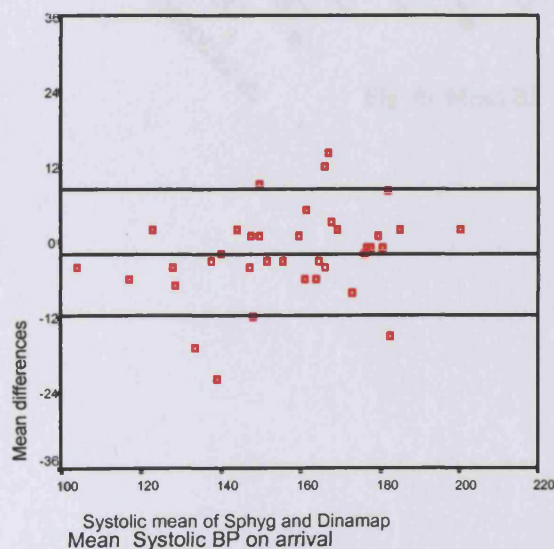


Fig 2a Systolic

Figure 2 Bland Altman analysis of Sphygmomanometer and Dinamap systolic (2a) and diastolic (2b) readings. Reference lines indicate the mean difference of the two methods and their limits of agreement (± 2 SE from the mean differences).

Figure 3 Error bars showing blood pressure variability of readings during the dialysis cycle for systolic (3a) and diastolic (3b) (limits indicate 95% confidence intervals).
Con av = conceptual average BP Key to abbreviations refer to legend Table4.

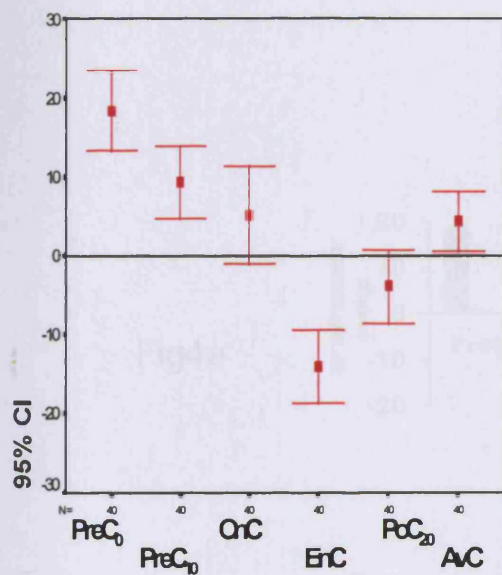


Fig 3a Systolic BP

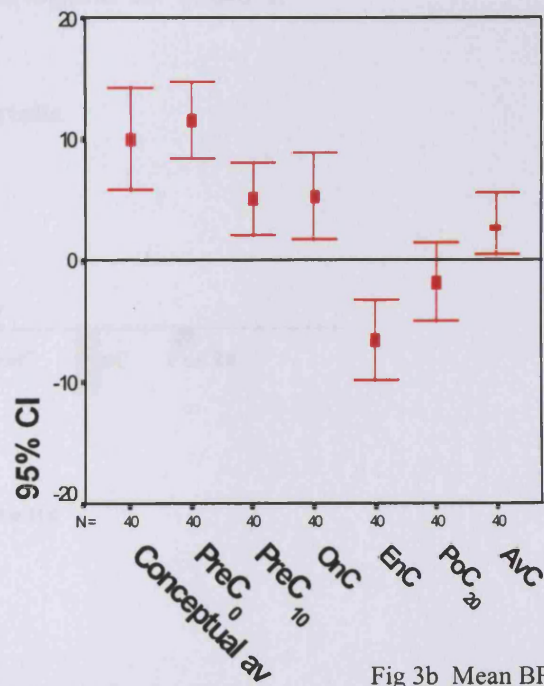


Fig 3b Mean BP

Figure 4 Bar diagram demonstrating the mean differences of the blood pressure measurements during the dialysis cycle from the interdialytic mean (0 on Y axis) for systolic(4a), diastolic(4b) and mean (4c) blood pressure. Key to abbreviations refer to legend on Table 4.

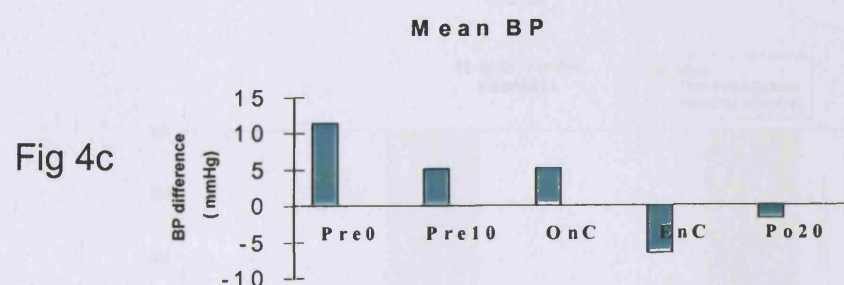
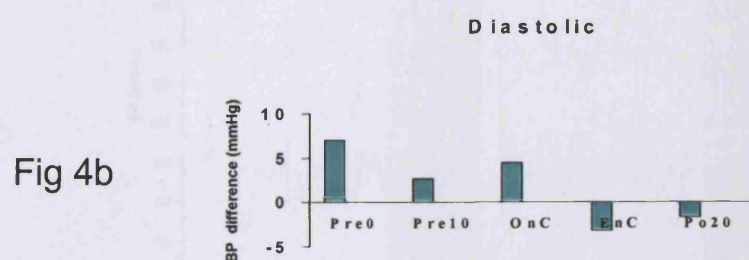
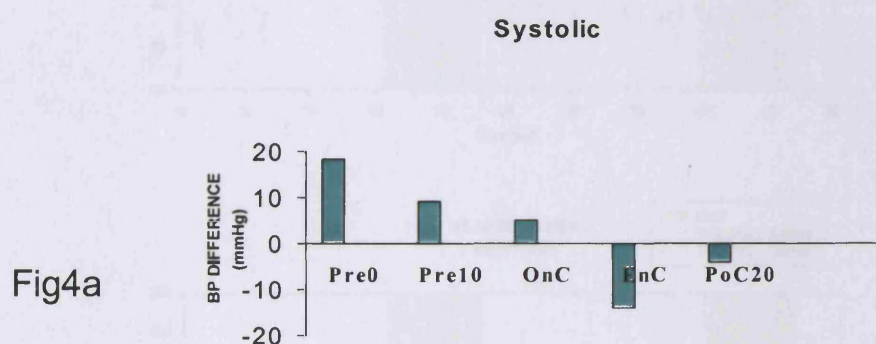


Fig 5a

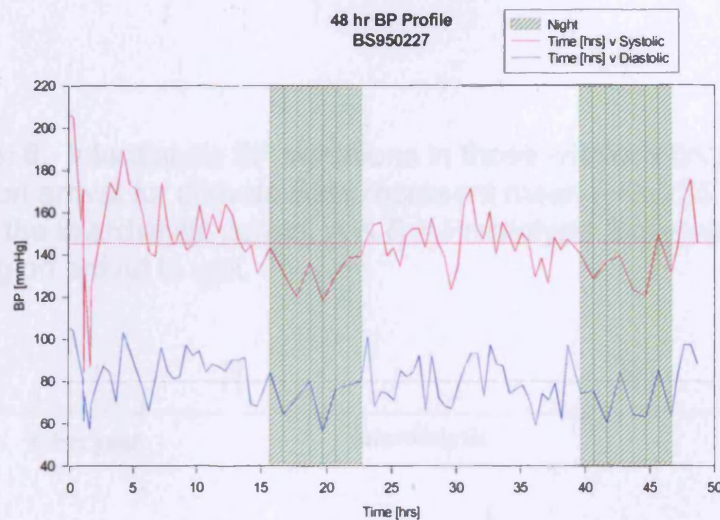


Fig 5b

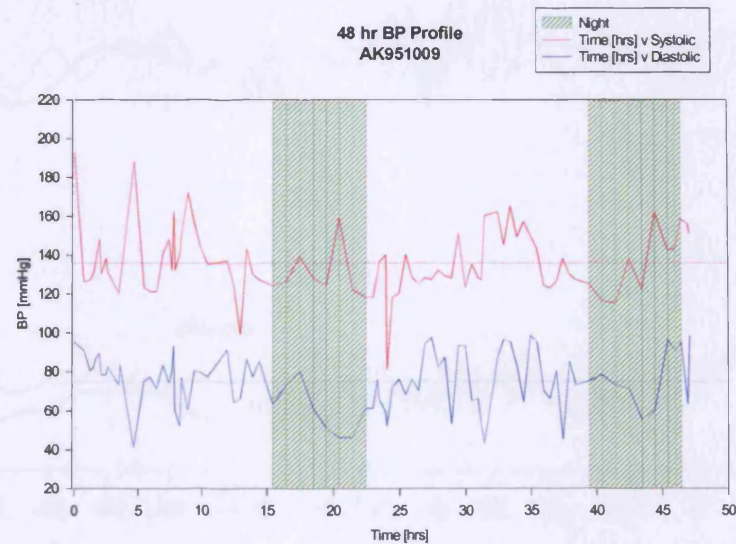
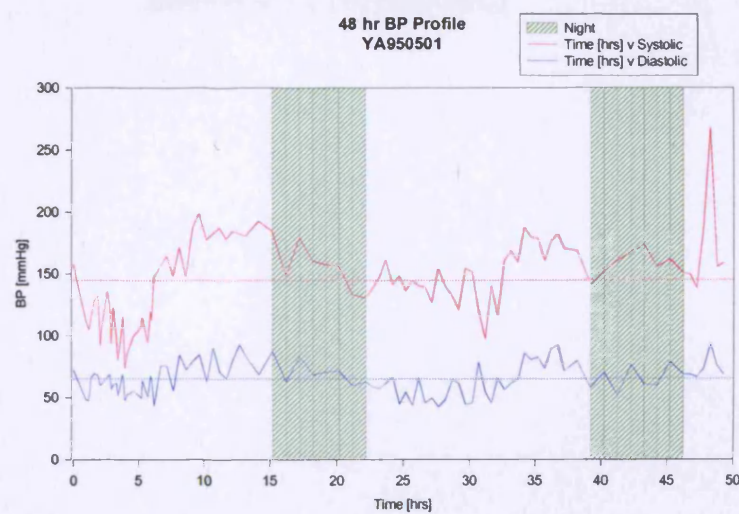


Fig 5c



Case Studies Figure 5 Illustrations of three patients on ABPM monitoring
5A Dipper 5B Nondipper 5C Diabetic nondipper with white coat effect
predialysis and frequent dialysis hypotension.

Figure 6. Interdialytic BP variations in those with or without white coat effect on arrival for dialysis. Bars represent mean (\pm 2SE) BP readings during the interdialytic period. A & B = Predialysis Sphygmomanometer reading on arrival to unit

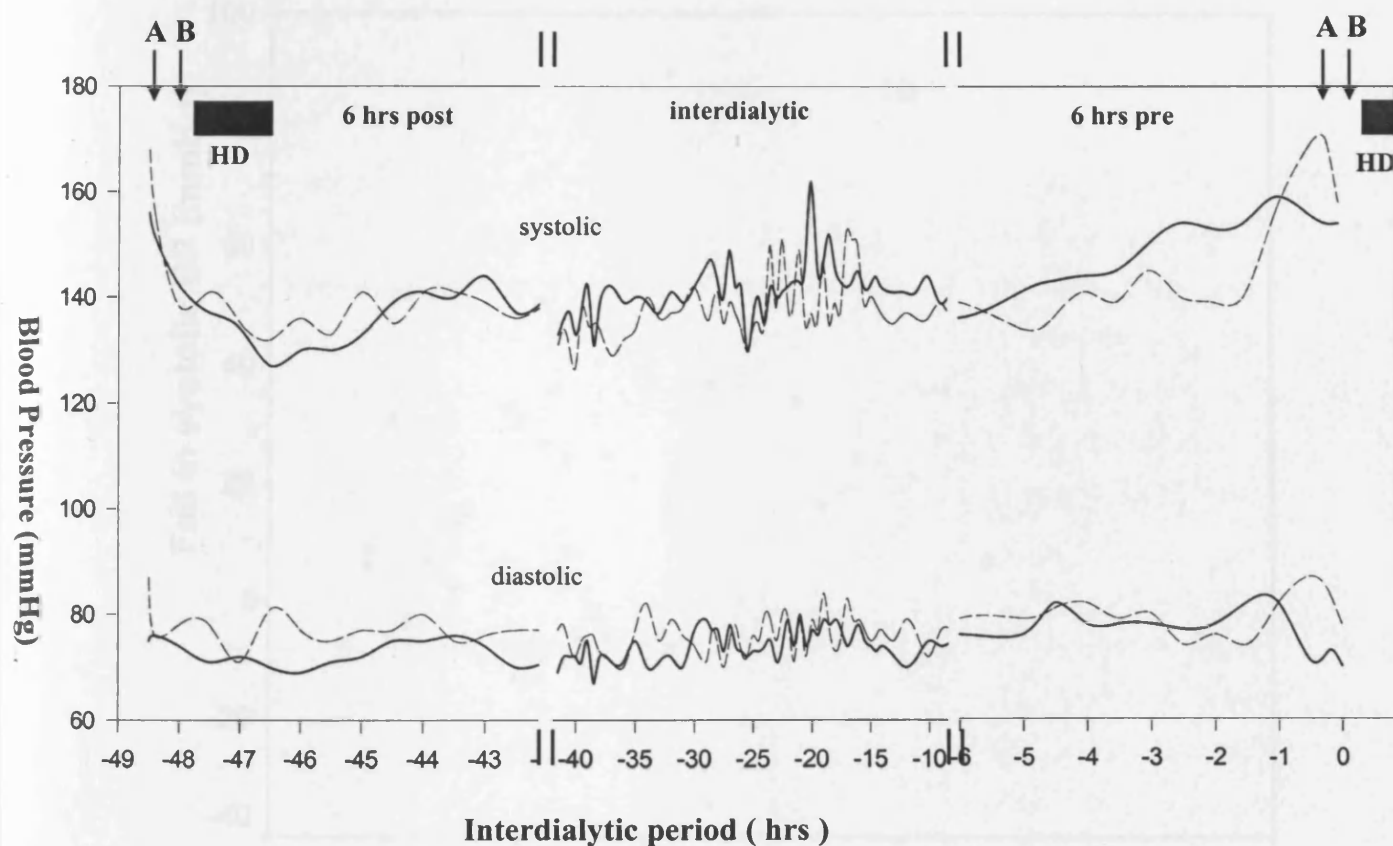
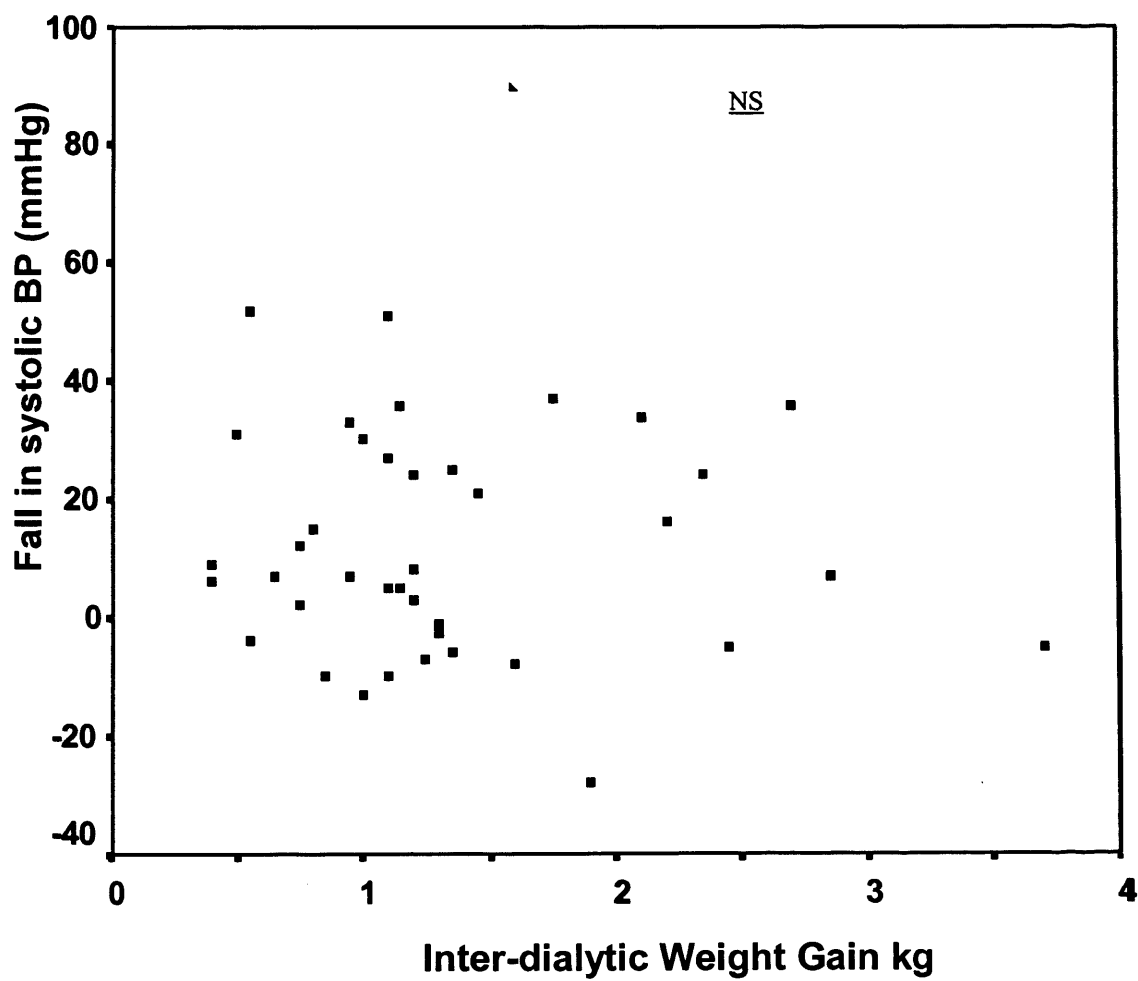


Figure 7 The poor relationship between the systolic BP drop and interdialytic weight gain



Chapter 2

The assessment of body composition and fluid compartments

Introduction

Approximately 60% body weight is water in normal individuals 30 – 40% of which is extracellular water (ECW). Sodium and water homeostasis is abnormal in haemodialysis (HD) patients, however the distribution of excess fluid has not been characterized [194] Despite best efforts, many patients are admitted with fluid overload and after hospital admission patients often achieve a new lower dry weight. The less accessible fluid compartments i.e. interstitial compartment harbors much of the excess fluid, influence plasma refill and more importantly reflect the hydration status of the patient.

Body weight at euvolemia also reflects the nutritional status of the dialysis patient. The latter may be compromised in chronic renal failure. [195] Therefore both variations in fluid and nutritional status may have direct consequences on dry weight variation and confound assessment of postdialytic target weight. Although various measures of nutritional status and clinical markers of hydration exist, adequate information on both body composition and fluid compartments cannot be obtained using a single measurement. [196] Wide limits of agreement were found between various techniques used to assess body composition in PD patients. [197] The assumption that derived indices such as kinetically estimated protein catabolic rate (PCR) generally reflect dietary protein intake (DPI) and thereby, indirectly, patient's nutritional status, has also been questioned. [198]

The aim of this clinical study was to compare different techniques used to evaluate body composition and to assess the influence of fluid status on the assessment of body composition. Bioimpedance is a technique that can measure these fluid spaces in a noninvasive manner, assess the nutritional status and changes in body

composition. This technique was used to assess a) fluid compartment volumes and its shift during UF b) compare the nutritional status using bioimpedance estimates and other indices of nutrition in patients with varying degrees of chronic renal failure and on dialysis.

EXPERIMENTAL SETUP

Subjects and Methods

Four groups of patients were studied : Predialysis (serum creatinine > 400umol/l and creatinine clearance <20 mls/min), Haemodialysis (HD > 3 months), Peritoneal dialysis (CAPD > 3 months) and controls. Randomly selected subjects of varying age and sex with no known major illness or renal impairment were used as controls. Subjects were randomly selected from an outpatient's clinic for a cross-sectional prospective nutritional assessment to compare direct and modeled estimates of nutritional status in patients with renal insufficiency. The only exclusions were malignancy, treatment modality change or recent (within 2 weeks) hospitalization. The study was approved by the North Herts Ethical Review Committee. All patients gave informed written consent. Dietary interventions were minimal.

Each subject underwent :

- i) 24 hr (predialysis or controls) or 48hr (HD or CAPD) urine collection was performed for urea kinetic modelling [112] and calculation of nitrogen losses
- ii) nutritional survey by anthropometry (Anthr) (same dietician), [199]
- iii) 3-day weighted food intake
- iv) Subjective Global Nutritional Assessment (SGNA), [196]
- v) Multifrequency bioimpedance (Bio) to assess fat free mass, total body water volume and extracellular fluid volume (see Methods section) with Bioimpedance measurements in patients treated by PD made after drainage of CAPD fluid and in those treated by HD postdialysis. To assess fluid shifts during ultrafiltration, a

subgroup of twelve HD subjects had additional bioimpedance measurements performed before and after a single dialysis session. These patients were also monitored with respect to their relative blood volume and blood pressure pre and post dialysis (see methods). Measured postdialysis ECW was also compared against estimates from derived formulae $ECW = (5 * Weight \div 70)$. [78]

vi) Blood sampling for biochemical markers - C-reactive protein (CRP), serum bicarbonate, transferrin (Tf), albumin (Alb) prealbumin (Palb) and Insulin growth factor (IGF-1) assay. [200]

The results and analysis were divided into three sections:

- a. Assessing the fluid compartment volumes and their resistivities in patients with varying degrees of chronic renal failure and on dialysis (HD and PD)
- b. Tracking fluid shifts in the EC compartment during HD pre and post UF using bioimpedance analyzer.
- c. Determine and compare the nutritional status of the patients using bioimpedance and other indices of nutrition.

Results

Section A

Repeatability and reproducibility of bioimpedance measurements

Reproducibility of the measurements was examined by obtaining data in triplicate for ECW, ICW and TBW (n=167). (Table 1) The method was most accurate for ECW measurements (CV 1.1 ± 3.2 %). ICW showed more variation (CV 3.6 ± 4.4 %). The fit status was good or excellent in 94% measurements.

Hydration status in Controls and chronic renal failure

Hydration status in controls and subjects with varying degrees of chronic renal impairment based on Creatinine clearance groups of Ia ($25 - 50$ ml/min/ $1.73m^2$) Ib ($12-25$ ml/min/ $1.73m^2$) and Ic (<12 ml/min/ $1.73m^2$) are depicted in Table 2a & 2b.

Abnormal distribution of fluid was evident between ECW and ICW compartments in renal impairment as compared to controls. Advanced renal failure with a Creatinine clearance $< 12 \text{ ml/min/1.73 m}^2$ was associated with significant expansion in the ECF and an increased Bioratio of above 50%. The subjects had a higher mean age; the body weight was not significantly different between the groups. The ECW and TBW had a weak but significant correlation with dietary sodium intake ($r = 0.20$, $P < 0.05$). There was no relationship of serum albumin or urinary protein leak to ECF. Serum albumin however correlated significantly with ICW ($r = 0.27$, $P < 0.05$) and negative correlation to the Bioratio ($r = -0.28$, $P < 0.05$).

Hydration status in ESRF subjects on dialysis

There were no significant differences between the measurements in CAPD and postdialysis HD even when corrected for weight or fat free mass. (Table 3) Controls and HD subjects who had all valid fluid measurements ($n=40$) were compared with respect to their bioimpedance volume measurements corrected for their ideal body weight or Fat free mass. (Table 4) Although the patients were in the similar age ranges in the HD and control group there was a significant difference in their weight, albumin and volume of distribution thereby reflecting different body composition. When corrected for the weight and free fat mass, the HD group had a significantly higher ECW per kg post dialysis and total body water relative to the fat free mass despite being clinically judged to be at their dry weight. (Fig 1) This may reflect a different compartmental distribution of water in fluid compartments or insensitivity of clinical methods to determine hydration of the fluid compartments in this group. The relationship of the ECW and ICW to the total body water was most abnormal and reversed in the predialysis phase. This trend was partially corrected with dialysis. (Fig 2)

Comparing Resistivities

Under normal conditions the ratio of the intracellular to extracellular fluid volume is tightly regulated and alterations in the ratio can reflect either a change in the intracellular or extracellular volume. The resistance itself measured by the bioimpedance is a measure of the electrical properties of water compartment and thus

influenced by electrolyte composition, protein, haematocrit and temperature. The use of resistances alone avoids further assumptions of height and volume relationships in different populations or reliance on complex emulsion theory. The ratio R_i/R_e is therefore the equivalent to the volume ratio ECW/ICW. This resistance ratio is tightly distributed in the normal population.

Most dialysis patients and those with renal impairment had an elevated ratio suggesting extracellular volume overload. (Table 5) CAPD patients in general had higher ratios than HD although the differences however did not achieve significance. It is assumed in this analysis that the resistivities of ICW and ECW are same for controls and renal failure. It is also assumed that these remain unchanged during dialysis alone. An increase in ratio can also be due to a decrease in ICW.

Section B

Studies during HD with UF

Twelve subjects on HD had measurements taken before and after dialysis with intermittent UF. Table 6 All patients showed significant reduction of ECW as measured by Bioimpedance with a tendency to plateau towards the end of ultrafiltration. Fig 3 depicts reduction in ECW and ICW with UF in one subject. There is usually a tendency for the ECW changes to plateau near the end of UF. The mean UF volume removed and mean ECW change were not significantly different and strongly correlated to each other ($r^2 = 0.93$) (Fig 4) The ECW change underestimated weight change of UF in excess of 2 litres. ($r^2 = 0.76$) (Fig 5) This could be a result of truncal fluid shift, insensible losses or obligatory UF. The latter is most unlikely with volumetric apparatus. The measured ECW overestimated the predicted by 0.8 ± 1.54 l ($r=0.79$ $p<0.001$). Fig 6 Two patients became hypotensive in the last hour and required saline bolus Subject 2 & 12. The ECW failed to pick up the infusion change accurately and immediately postinfusion perhaps due to lack of equilibration.

The effective change in blood volume at the end of dialysis was strongly correlated with the reduction in weight ($r=0.62$), ECW ($r=0.68$) and UFV removed ($r=0.70$).

$p < 0.05$. Systolic BP reduction was predicted by the reduction in blood volume, weight and ECW change. These results emphasize the fact that the volume shifts in the ECW compartment are major determinants of haemodynamic stability.

Section C

Nutrition and Extracellular water

Eighty patients (pts) were recruited for this part of the study, with varying degrees of chronic renal impairment (CRF) (Gr I), 79 stable ESRD pts (CAPD (Gr II) $n=39$; HD (Gr III) $n=40$) and 33 controls (Gr IV) (Table 7). Mean weekly Kt/V was 2.3 ± 0.49 in CAPD and 3.9 ± 0.59 in HD. Mean (\pm SD) ages were 44 ± 13 , 59 ± 14 , 61 ± 11 and 56 ± 15 yr. for controls, CRF, CAPD and HD pts respectively. In the control and CRF subjects, malnourishment (SGNA) was evident in 5.8% in Gr Ia ($\text{CrCl} > 50 \text{ ml/min/m}^2$; $n=34$), 5.8% in Gr Ib ($\text{CrCl} 25\text{-}50 \text{ ml/min/m}^2$; $n=34$), 13.8% in Gr Ic ($\text{CrCl} 12\text{-}25 \text{ ml/min/m}^2$; $n=29$) and 50% in Gr Id pts ($\text{CrCl} < 12 \text{ ml/min/m}^2$; $n=10$) (Fig 7). nPCR correlated with measured protein intake wDPI ($r=0.46$, $p < 0.01$). nPCR significantly decreased with fall in CrCl in each group, (Fig 8) but the mean FFM (Anthr), FFM (Bio) and wDPI decreased significantly in Gr Id only (Fig 9) (Table 8). Creatinine clearance corrected for body size (CrCl ml/min/m^2) correlated positively with nPCR, Calculated protein intake (cDPI), Protein nitrogen appearance corrected for ideal body weight ($\text{ht}^2 \times 22.5$), 24hr creatinine excretion, Alb, Tf and IGF-I ($p < 0.01$) and negatively with age ($p < 0.01$) and CRP ($p < 0.05$) but not with bioimpedance, wDPI or anthropometric indices. Albumin was correlated with Tf, IGF-I (Fig 10, 11) and negatively with CRP ($p < 0.01$). CRP was strongly predicted by bicarbonate and Alb. (Fig 12) Palb and IGF-I were not significantly different between groups. (Fig 13) Calculated DPI (y) was less precise and underestimated the wDPI (x) (Fig 14) ($R = 0.44$ $R^2 = 0.19$, $p < 0.01$) ($y = 0.47 + 0.5x$; $r^2 = 0.2$). A higher proportion of HD patients (40%) were malnourished (SGNA) than CAPD (28%), CRF (14%) and controls (6%). (Fig 11) Skinfold thickness (SFT), fat mass (FM), cholesterol, Palb, and IGF-1 were higher in CAPD, mid-arm circumference, Tf levels similar, Fat free mass FFM (Anthr) & FFM (Bio), CRP and nPCR were lower

($p < 0.01$) than HD pts. (Fig 15) SGNA scores were not significantly different between those on dialysis for >1 yr. or less (CAPD $n = 30$ v 9 & HD $n = 27$ v 13) despite similar weekly KT/V (CAPD 2.27 v 2.45 ; HD 3.89 v 3.86).

Discussion

Fluid compartments

A stable volume and composition of extracellular fluid are essential for normal functioning of the body. Since the kidney is primarily responsible for regulating extracellular fluid, loss of kidney function should have catastrophic consequences. Fortunately, even with loss of more than 90 percent of renal function, a remarkable capacity to regulate body fluid volumes and sodium and potassium persists. Nevertheless, this capacity is limited to chronic renal disease and this has important consequences for clinical management of these patients.

Bioimpedance spectroscopy or multiple frequency bioimpedance analysis has become a widely used technique in body composition analysis in recent years. Alternating current at a low frequency flows through ECF while high frequencies current flows through total body fluids. Using a spectrum of measuring frequencies, typically 5 – 500 Hz purports to assist in the discrimination between extracellular and intracellular fluid.

The results suggest dialysis patients presumed to be euvolemic on clinical grounds, may remain above their true physiologic dry weight defined as normal hydration of body tissues. Abnormally elevated Bio Ratio and Ri/Re ratio are indicative of the phenomenon. This is supported by the fact that following transplantation subjects often achieve a weight lower than the dialysis dry weight.

Despite its limitations the Ri/Re ratio can be a useful marker of tracking fluid status. Elevated Ri/Re ratio should prompt a search for extracellular volume overload. Spiegel et al demonstrated elevated Ri/Re ratio could be due to loss of intracellular volume in malnourished patients. Urea, which fluctuates on dialysis, does not affect

resistivity [201]. Excess fluid accumulating in the central compartments is not measured by limb resistivities. Excess fluid is often held in mesenteric, splanchnic and hepatic and pulmonary veins. Bioimpedance is insensitive to changes in the central fluid compartments. Hence a normal ratio cannot exclude an excess body water in central compartments.

There are several other potential errors that can affect bioimpedance measurements. First the resistance itself, a measure of electrical properties of water compartment and thus influenced by electrolyte composition, protein concentration, haematocrit and temperature. The conversion of resistance to volumes requires a further assumption based on height and volume relationships determined in different populations or a reliance on emulsion theory. In intensive care fluid balance studies atypical impedance values were observed compared with those previously seen in normals with wide variations from day to day and impedance plots. Stray capacitance in the environment, interaction between electrode impedance and lead positioning are factors. [202]

Dietary sodium intake influences the volume of the extracellular compartment in all groups of patients with renal dysfunction. The successful management of hypertension in patients undergoing maintenance haemodialysis depends primarily on the control of total body sodium. [65] Sodium forms the principle backbone of the ECW, which expands with salt and water retention. Restricting sodium intake is likely to help control hypervolemia in patients with renal dysfunction. Many patients with advanced renal disease may be extremely sensitive in terms of blood pressure elevation and to sodium overload as demonstrated in renoprival dogs [203] and by constant and careful regulation of total body sodium control can satisfactory BP control be maintained. The progressive decline in residual renal function is likely to exacerbate the expansion of the ECF due to salt retention and abnormal salt sensitivity.

In the long term management of HD patients a continuous normal level of BP can be maintained if adequate sodium restriction can be maintained between dialysis. Haemodynamic stability and blood volume changes had a significant relationship with ECW. Though it is possible to reduce the ECW to normal levels acutely by

ultrafiltration, in many patients there may exist a lag time between reduction of ECW and adequate control of BP. [70]

The nutrition overlap

The findings in this study indicate that malnutrition is a late phenomenon in advanced renal insufficiency with a significant decline in nutritional state just prior to dialysis. (Fig 9) These remain only partially corrected on dialysis with a high prevalence despite adequate modelling parameters. [204] nPCR estimates are likely to be artefactual partly due to mathematical coupling with CrCl estimates or errors in urine collection. [205] Plasma proteins and serum albumin though strong predictors of adverse outcome in CRF are insensitive to the presence of earlier stages of malnutrition and may be affected by abnormal metabolism in CRF. Visceral protein markers may also be independently affected by continuing catabolic insults possibly due to changing renal function or dialytic process itself. Malnutrition in uremic subjects coexists with ECW expansion. (Fig 6)

The body composition in advanced renal failure is abnormal which makes interpretation and early detection of malnutrition difficult. The assessment of body composition was strongly influenced by hydration state. This problem is confounded by fluctuating fluid status. Reliable detection and adequate treatment of malnutrition in severe renal impairment poses a major challenge. Bioimpedance technique whilst measuring fluid compartments with reasonable accuracy, can also incorporate lean body mass characteristics reflecting nutritional status. An objective assessment of the extracellular compartment in conjunction with the nutritional status with the use of such tools will enable better fluid and nutritional management.

Table 1 Reproducibility of triplicate measurements (M1,M2,M3) & between left and right sided measurements (Rt, Lt)

	R (M1, M2)	R (M2, M3)	CVmean (%)	CVsd (%)	R (Rt, Lt)
ECW (Rt)	0.98	0.99	1.1	3.2	0.98
ECW(Lt)	0.99	0.98	0.6	3.1	
TBW (Rt)	0.94	0.97	2.0	4.5	0.95
TBW (Lt)	0.99	0.94	1.9	6.6	
ICW (Rt)	0.95	0.99	3.6	4.4	0.88
ICW (Lt)	0.98	0.92	2.9	4.9	

Rt right side, Lt Left side R = Correlation CV = method coefficient of variation

Table 2a Body fluid distribution (Controls)

N	33
Creatinine clearance ml/min/1.73m ²	102 ± 28.7
Weight (kg)	90.8 ± 19.4
Surface Area m ²	1.96 ± 0.29
Body mass index	28.6 ± 5.4
ECW Litres	21.4 ± 3.98
TBW Litres	50.3 ± 12.5
ICW Litres	28.9 ± 9
ECW L/kg	0.24 ± 0.03
ECW/ FFM L/kg	0.34 ± 0.03
TBW L/kg	0.56 ± 0.01
ICW L/kg	0.32 ± 0.08
ECW/TBW (Bioratio)	0.44 ± 0.05

ECW = Extracellular water ICW Intracellular water

TBW Total body water FFM Fat free mass

Table 2b Body fluid distribution in subjects with chronic renal impairment

Parameters	CrCl (25 –50)	CrCl (12-25)	CrCl (< 12)
N	34	29	10
Creatinine clearance ml/min/1.73m ²	36.5 ± 7	17.4 ± 3.3**	9.5 ± 2.7**
Weight (kg)	79.7 ± 14.1	82 ± 17.6	76.1 ± 14.8
Age yrs	59.8 ± 14.6	59.3 ± 14.6	67.5 ± 9.9*
Albumin	37.7 ± 4.5	38.3 ± 3.5	35.1 ± 4.7
Surface area m ²	1.95 ± 0.21	1.98 ± 0.25	1.88 ± 0.22
Body mass index	27.8 ± 4.1	29.4 ± 5.7	27.7 ± 4.65
ECW L	18.5 ± 4.1	20.2 ± 2.4	20.8 ± 4.5*
ICW L	18.4 ± 3.2	22.3 ± 4.7	20.4 ± 6.4
TBW L	37.4 ± 6.4	42.4 ± 7	41.2 ± 10.4
ECW/TBW (Bioratio)	0.47 ± 0.04	0.47 ± 0.03	0.51 ± 0.05*
ECW L/kg	0.28 ± 0.03	0.29 ± 0.04	0.31 ± 0.04
ICW L/kg	0.25 ± 0.05	0.23 ± 0.04	0.2 ± 0.04
TBW L/kg	0.52 ± 0.07	0.52 ± 0.07	0.52 ± 0.07
ECW/FFM	0.41 ± 0.03	0.4 ± 0.03	0.45 ± 0.03*

ECW = Extracellular water ICW Intracellular water TBW Total body water FFM Fat free mass

CrCl Creatinine clearance ml/min/1.73m² *p<0.05, ** p< 0.01 compared with Column 2

Table 3 Comparison of body fluid composition measurements between peritoneal (CAPD) and haemodialysis (HD)

Parameters	CAPD	HD
N	39	40
Age	61.7 \pm 10.6	55.6 \pm 15.3
Weight (kg)	74.5 \pm 7.7	66.9 \pm 13.9
BSA m ²	1.8 \pm 0.15	1.8 \pm 0.24
BMI	26.1 \pm 3.9	25.2 \pm 5.5
ECW L	18.5 \pm 3.2	17.4 \pm 3.3
TBW L	38.3 \pm 6.1	36.1 \pm 7.6
ICW L	20.1 \pm 3.9	18.9 \pm 5
ECW/TBW	0.48 \pm 0.05	0.48 \pm 0.05

(No significant differences seen for any parameter)

Table 4 Fluid Compartment volumes in HD subjects measured postdialysis against controls

	Controls (33)	HD (40)	P value
Age yrs*	43.5 \pm 15.7	55.6 \pm 15.3	P<0.05
Weight kg*	93.2 \pm 21	66.9 \pm 13.9	P< 0.001
Ideal wt kg*	70.6 \pm 7.7	62.1 \pm 5.3	P< 0.01
Albumin g/dl*	40.8 \pm 3.5	35.8 \pm 4.3	P< 0.01
Watson V Litres*	47 \pm 8.8	36 \pm 6.1	P< 0.01
ECW*	21.5 \pm 4	17.4 \pm 3.3	P< 0.01
ICW*	28.2 \pm 9	18.8 \pm 5	P< 0.01
FFM*	63.6 \pm 14.7	50.2 \pm 9.4	P<0.05
ECW/wt*	0.23 \pm 0.03	0.26 \pm 0.02	P< 0.01
ECW/FFM	0.34 \pm 0.03	0.37 \pm 0.03	P=0.05
ICW/wt	0.3 \pm 0.08	0.28 \pm 0.05	P=0.42
ICW/FFM	0.43 \pm 0.39	0.39 \pm 0.05	P=0.09
TBW/wt	0.54 \pm 0.11	0.54 \pm 0.06	P=0.94
TBW/FFM*	0.77 \pm 0.01	0.76 \pm 0.02	P<0.05

Expanded ECW adjusted for body size measured postdialysis at the clinical dry weight suggests abnormal distribution. (* indicate p<0.05)

Table 5 Resistivity in the different groups of renal failure mean (sd)

	Re	Ri	DA	Cm	Ri/Re
Controls	526(88)	983(139)	24.4(1.3)	2.3(0.55)	1.89(0.26)
CRF	515(92)	1008(197)	24.6(1.7)	2.2(0.58)	1.98(0.31)
HD	546(95)	1090(255)	25.3(2.4)	1.93(0.74)	2.05(0.62)
CAPD	561(70)	1150(218)	25.2(1.1)	1.75(0.57)	2.08(0.46)

Table 6 Reduction in ECF during ultrafiltration in 12 HD subjects[(-) means a reduction]

Subject	Reduction in RBV %	UFV Litres	Change in ECF litres	Change in weight Kg	Predicted Post ECF litres	Measured Post ECF litres	MAP change mmHg	Systolic change mmHg
1	-9.0	-2.8	-3.02	-3.2	23.05714	22.8	-27	-34
2	-0.4	-0.8	-1.05	-0.85	12.42857	12.6	-21	-28
3	-12.6	-2.4	-2.1	-2.6	14.57143	13.8	-27	-37
4	-11.8	-2.57	-2.45	-2.9	21.38571	21.41	-20	-28
5	-7.9	-2.217	-1.7	-2	15.21429	18.02	-3	-11
6	-9.1	-1.978	-2.29	-2.5	17.74286	18.97	-11	-17
7	-13.9	-2.72	-2.67	-2.7	19.86429	22.68	-44	-68
8	-9.0	-1.8	-1.81	-1.8	13.99286	20.07	17	25
9	-4.1	-1.05	-1.29	-1	11.57143	14.73	5	12
10	-7.4	-0.8	-0.64	-0.8	13.99286	18.57	1	18
11	-17.5	-2.9	-2.52	-3.0	16.39	16.45	-31	-41
12	-11.7	-3	-3.11	-2.1	17.87143	16.88	-21	-29
Mean	-9.5	-2.1	-2.1	-2.1	16.5	18.1	-15.2	-19.8
STdev	4.5	0.8	0.8	0.9	3.6	3.3	17.4	27.0

Table 7 Baseline characteristics of subjects and subgroups

	CRF	Controls	HD	CAPD
Numbers	80	33	40	39
Age (yrs)	59(14)	44(13)	56(15)	61(11)
KT/V(weekly)	7.9(4.5) *	29.6(8)	3.9(0.6) *	2.3(0.5) *
IBW (kg)**	63.5(6.8) **	65.3(7.7)	62 (7.3) **	61.6(7.1) **
BSA (m²)	1.94(0.23)	1.96(0.29)	1.8(0.25)	1.8(0.16)
BMI	28(4.7)	27(5.1)	25(5.5)	26(3.9)

mean values (sd) * p (<0.05) ** p (<0.01)

Table 8 The nutritional indices compared in groups with varying degrees of renal dysfunction

	Gr Ia	Gr Ib	Gr Ic	Gr Id
nPCR (g/kg/d)	1.2(0.2)	1.0(0.2)*	0.89(0.2)*	0.74(0.1)*
wDPI (g/kg/d)	1.37(0.38)	1.2(0.3)	1.3(0.48)	0.98(0.15)*
FFM(Anthr) kg/1.73m ²	50.6(5.4)	49.7(5.2)	49.6(4.4)	45.8(4.7)*
FFM(Bio) kg/1.73m ²	50.6(7.9)	50.7(7.3)	50.69(6.9)	44.4(7.9)*
Albumin g/dl	40.8(3.4)	37.7(4.5)*	38.3(3.57)	35.1(4.7)*
Transferrin g/l	2.47(0.4)	2.24(0.3)	2.1(0.4)	1.87(0.23)*

mean values (sd)* p (<0.05) compared with Gr Ia Anthr = anthropometry, Bio = Bioimpedance wDPI weighed protein intake nPCR = normalised protein catabolic rate

Figure 1 Comparing mean ECW normalised to body weight and fat free mass between healthy controls and haemodialysis subjects

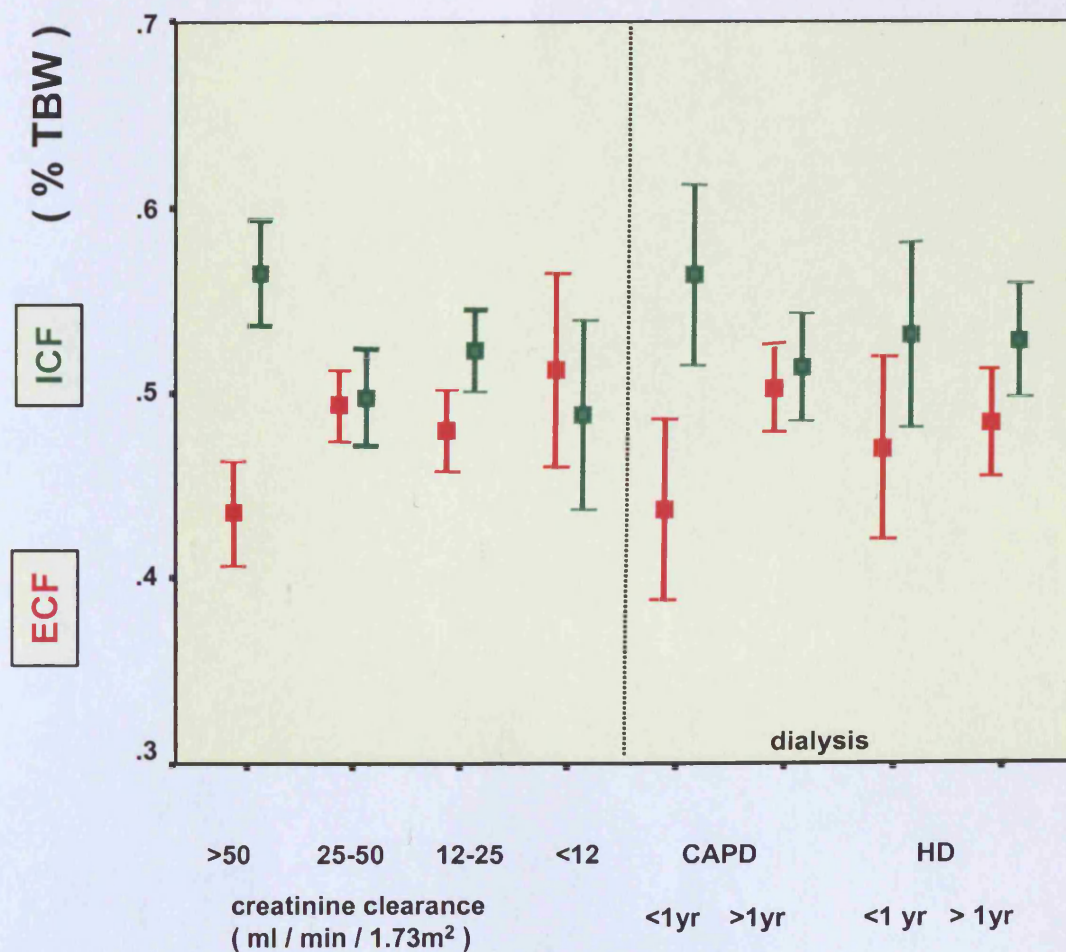
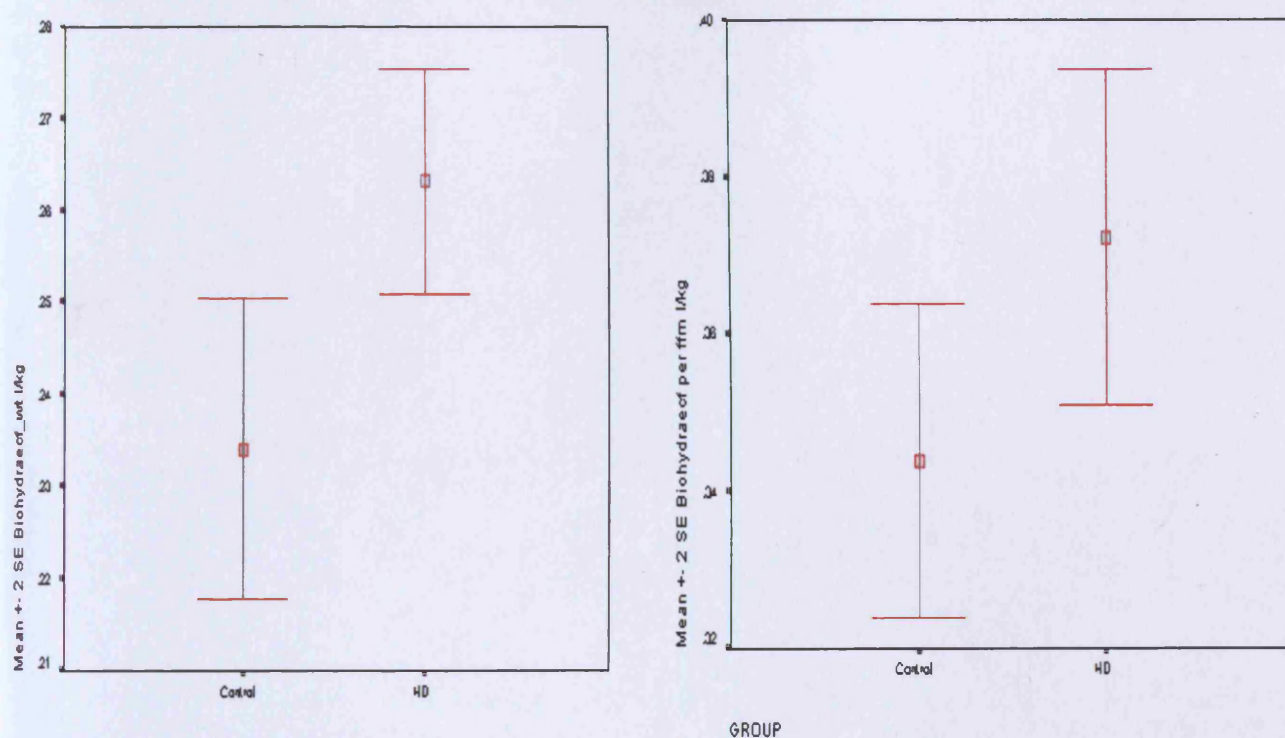


Figure 2 Malnutrition in uraemia is associated with ECF expansion

Figure 3 Typical changes in ECW (circle) and ICW (triangles) measured by bioimpedance during UF in a subject
(UF volume -2.4 L Wt change -2.6 L Post ECW 13.8 L ECWchange -2.1 L)

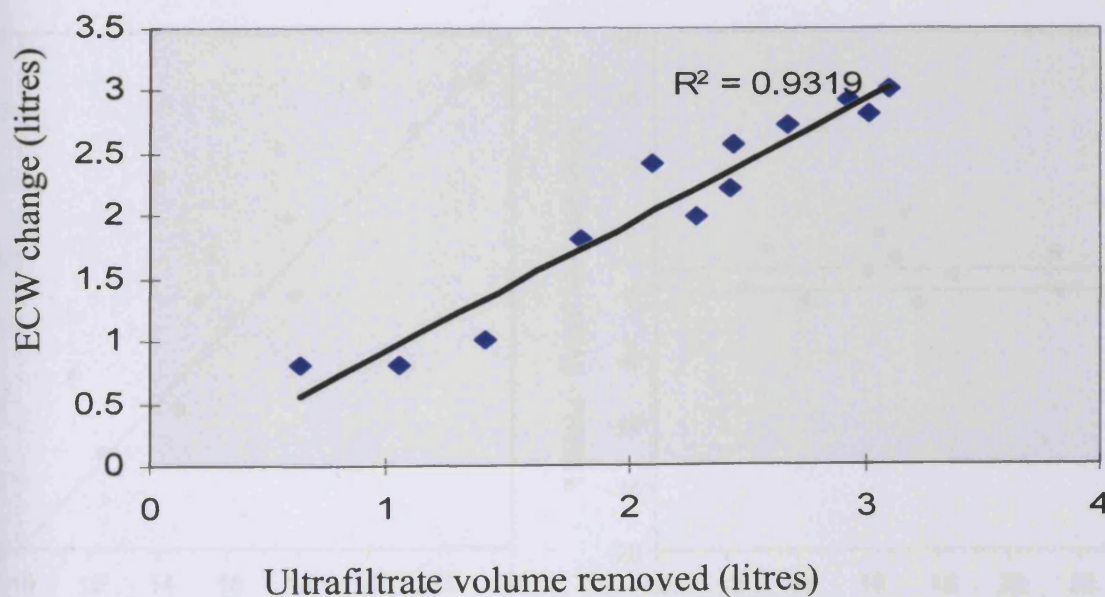
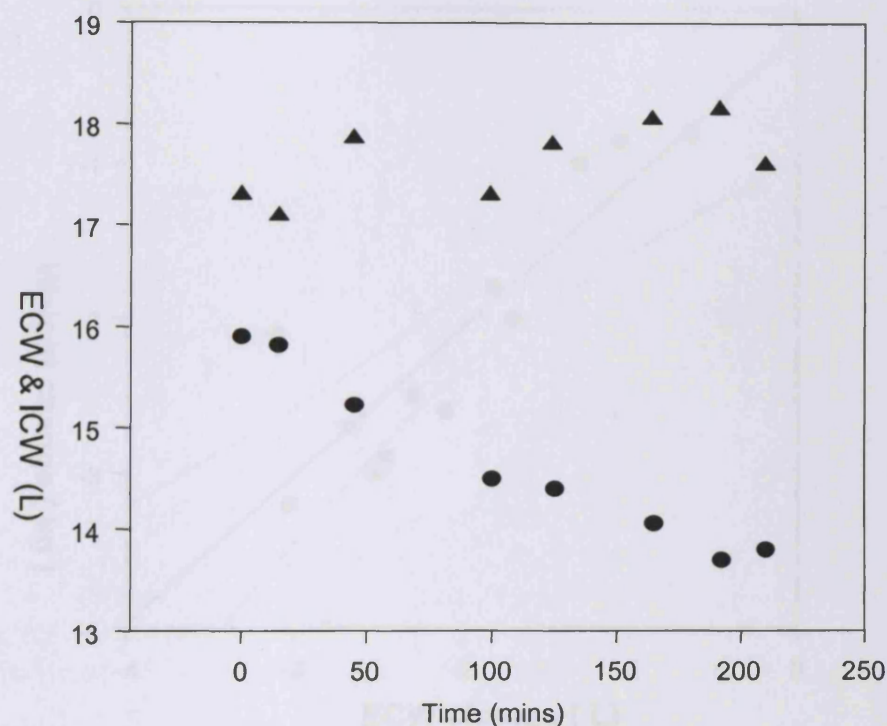


Figure 4 Bioimpedance tracks ultrafiltrate volume removed during HD
($R^2 = 0.93$)

Figure 5 Bioimpedance tracks body weight changes during HD ($R^2 = 0.76$)

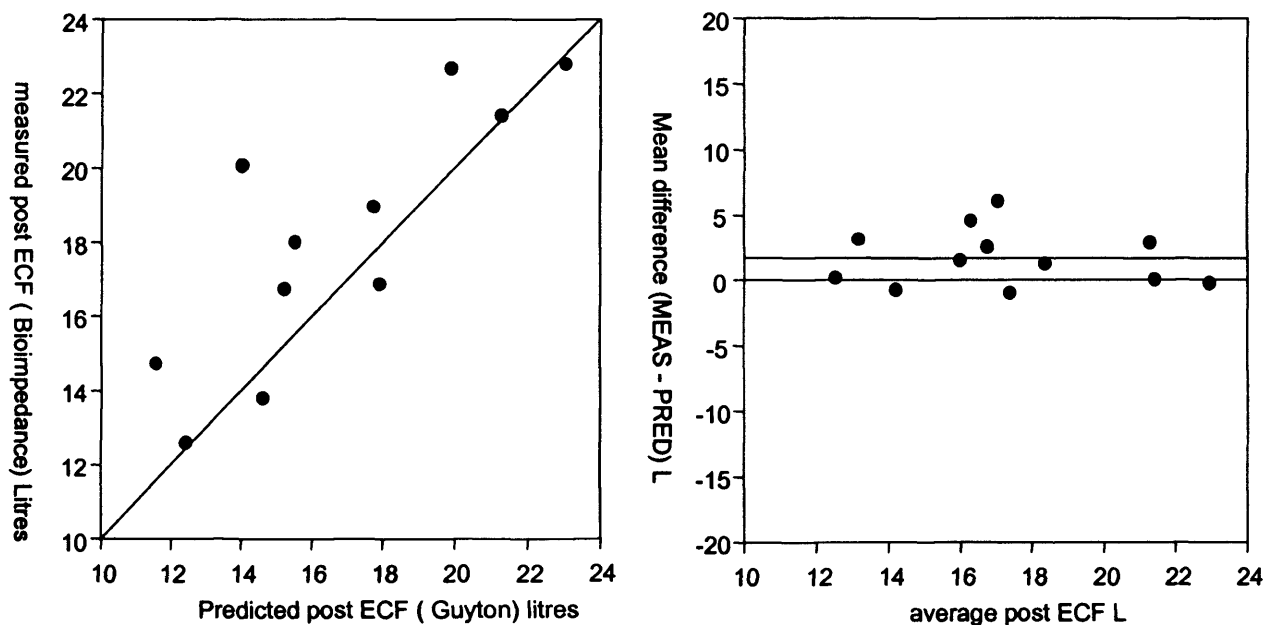
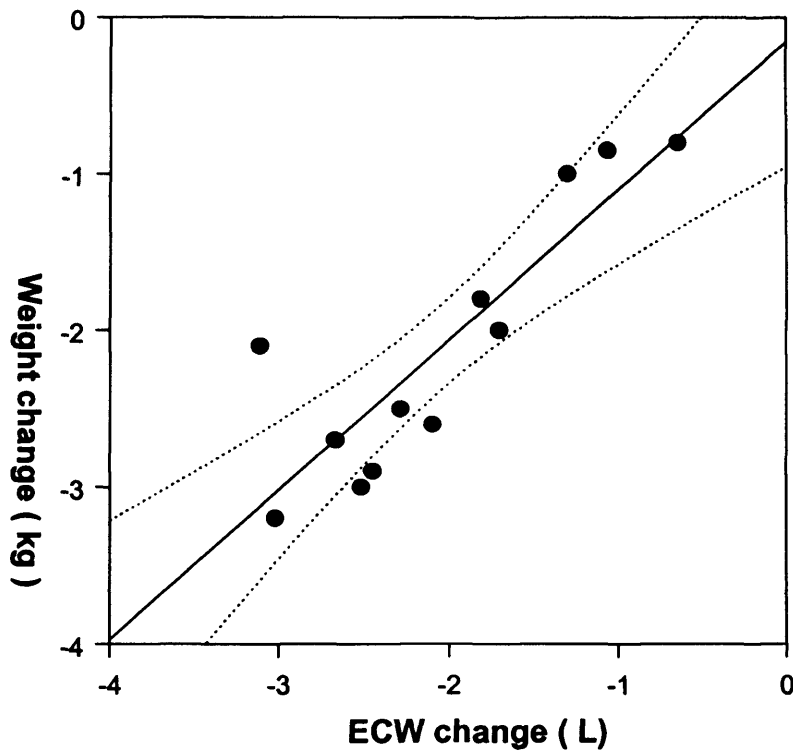


Figure 6 Bioimpedance measured post ECF vs predicted post ECF (Guyton)
 (Dotted line represents line of identity) ($p=0.02$ paired ttest)
 Mean difference of measured ECW 1.67 L

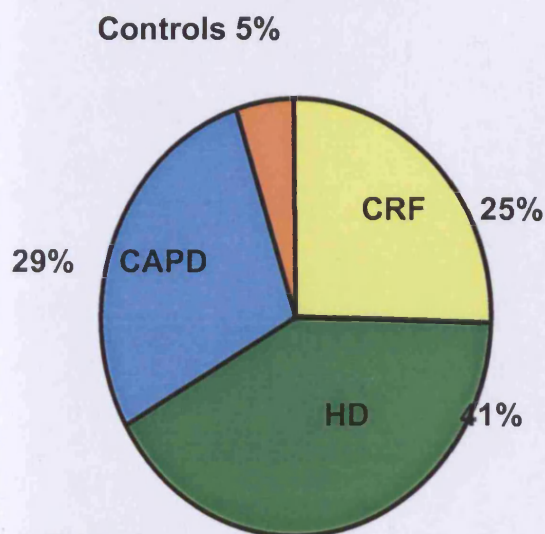


Fig 7a

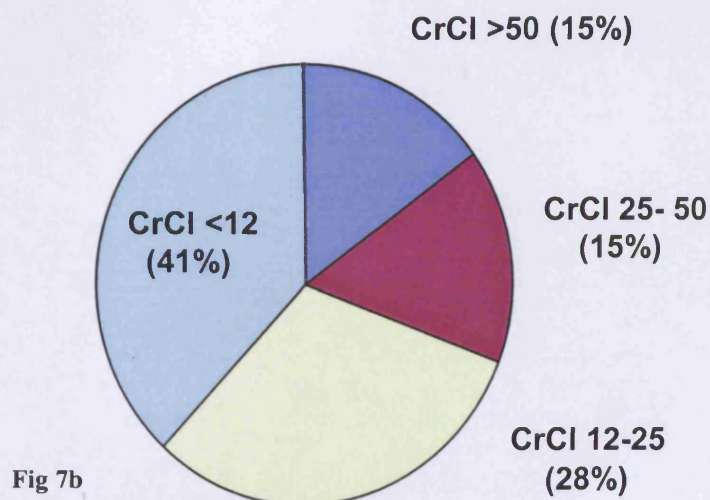


Fig 7b

Figure 7 Subjective Global Assessment of Nutritional Status (SGNA)

Proportion of Malnourished subjects (SGNA B & C) between groups (Fig 7a) & within nondialysis CRF group (Fig 7b) CrCl = creatinine clearance ml/min/1.73m²

Single observer SGA rating

A = Well nourished B = Moderately malnourished C = Severely malnourished

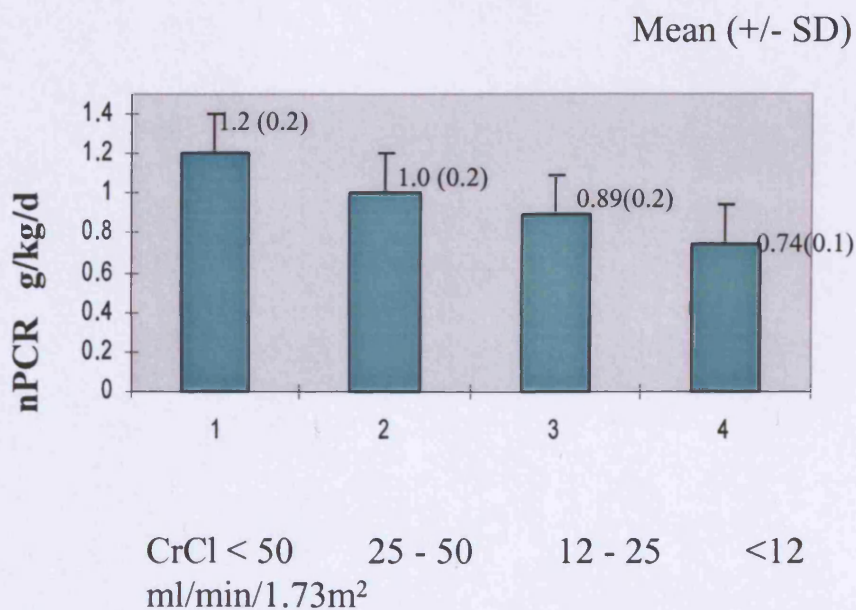
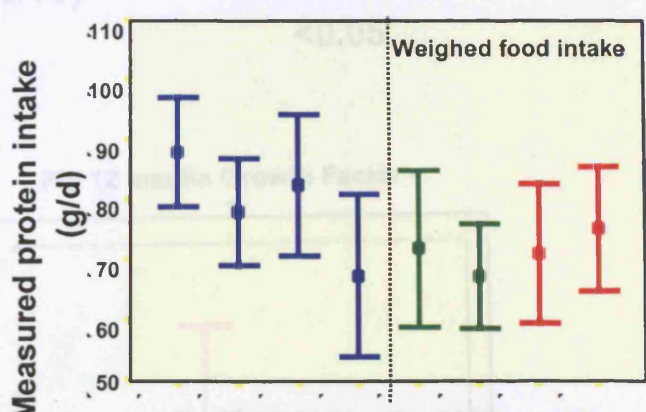
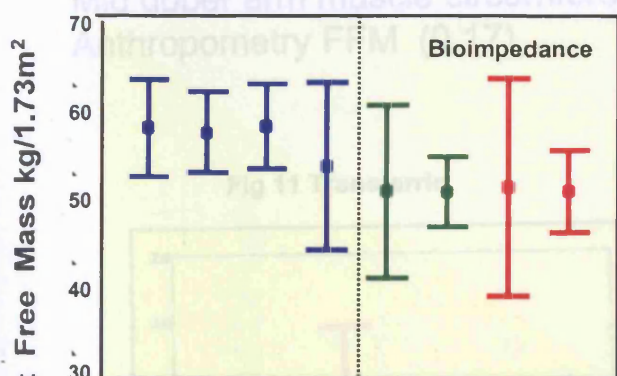
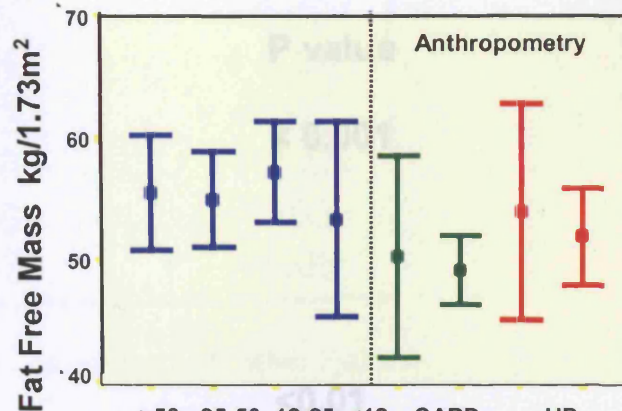
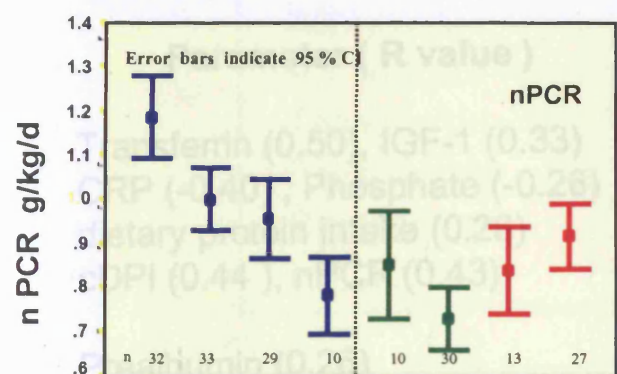


Figure 8 Protein catabolic rate nPCR (g/kg/d) correlated positively with creatinine clearance ml/min/1.73m² (r = 0.60 p < 0.001)

Figure 9 NUTRITIONAL STATE DECLINES JUST PRIOR TO DIALYSIS



Transferrin: BEST CORRELATES (R VALUE) $P < 0.01$

Albumin (0.5), CRP (-0.24), Prealbumin (-0.27)
 IGF-1 (0.3), Cholesterol (0.24), M/LAMC (0.24),
 SSK (0.31), nPCR (0.49), cDPI (0.4)

Albumin (0.33), transferrin (0.3)
 prealbumin (0.21), nPCR (0.22),
 cDPI (0.27)

Figure 10 Correlations of Albumin with other nutritional markers

Albumin & Other Nutritional markers

Significant
Correlations

Parameter (R value)

P value

Transferrin (0.50), IGF-1 (0.33)
CRP (-0.40) , Phosphate (-0.26)
dietary protein intake (0.28)
cDPI (0.44) , nPCR (0.43)

< 0.001

Prealbumin (0.26)
Mid upper arm circumference (0.22)

<0.01

Mid upper arm muscle circumference (0.18)
Anthropometry FFM (0.17)

<0.05

Fig 11 Transferrin

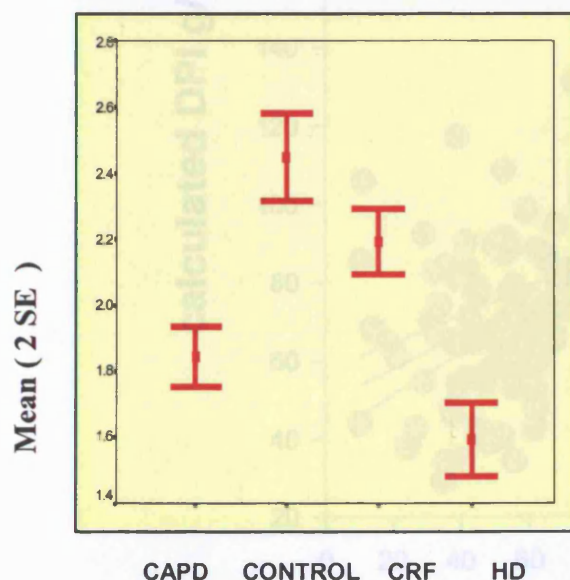
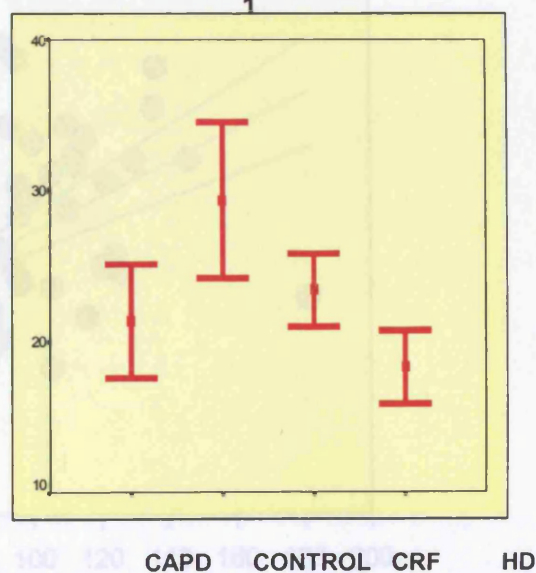


Fig 12 Insulin Growth Factor - 1



Transferrin BEST CORRELATES (R VALUE) P<0.01

IGF-1

Albumin(0.5), CRP(-0.24), Phosphate(-0.27)
IGF(0.3), Chol (0.23), MUAMC (0.24),
SSK(0.31) nPCR (0.30), cDPI(0.4)

Albumin(0.33) transferrin(0.3)
prealbumin(0.21), nPCR (0.22),
cDPI(0.22)

Significant Correlations with CRP

	R	P value
Albumin	-0.40	<0.001
Phosphate	0.26	< 0.001
Transferrin	-0.19	<0.01
Prealbumin	-0.19	<0.001
Bicarbonate	-0.18	<0.05
nPCR	-0.14	<0.05

Fig 13 C-Reactive protein and its correlation with other indices

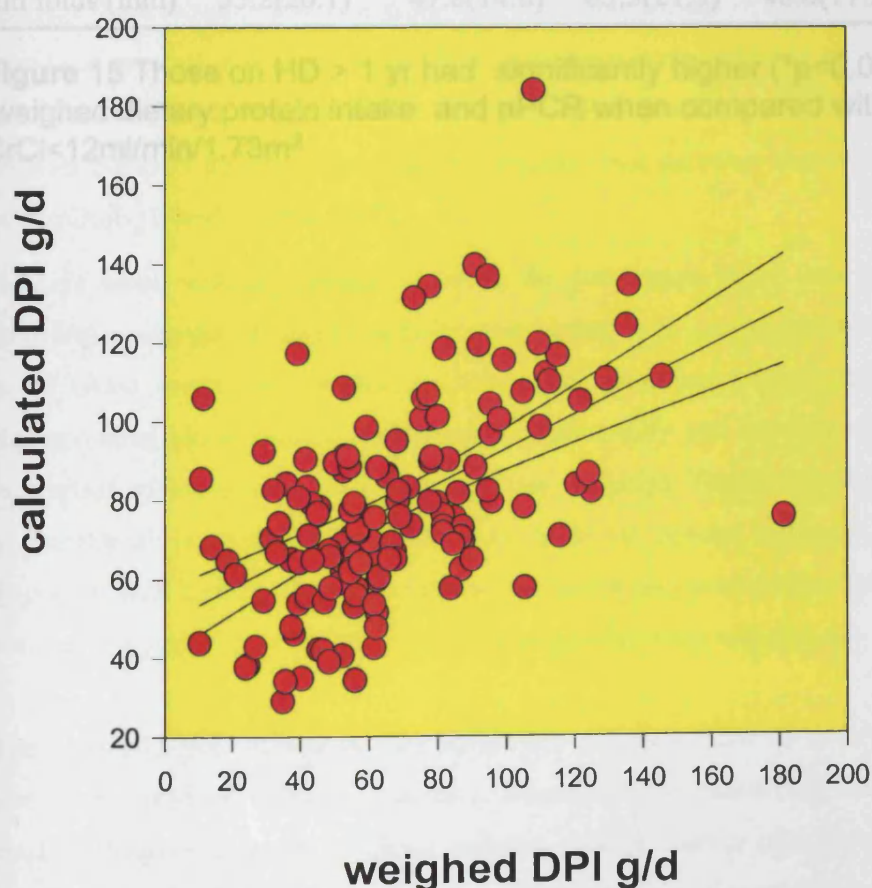


Fig 14 Dietary Protein intake (DPI)

Calculated DPI* Hakim et al (g/d) (y) underestimated the weighed DPI (x) ($y = 18.5 + 0.62x$; $r^2 = 0.27$ $R = 0.52$ $p < 0.001$).

Chapter 3

Relative blood volume measurements during Haemodialysis: Pilot studies

	CrCl <12	CAPD<1 yr	CAPD > 1yr	HD <1yr	HD>1 yr
FFM (Anthr) kg/1.73m ²	45.8(4.7)	48.2(5.6)	46.5(4.8)	50.4(5.5)	50.5(4.5)*
FFM (Bio) kg/1.73m ²	44.4(7.9)	48.7(7.3)	47.8(7.3)	49.9(8.7)	49.3(6.3)*
wDPI g/kg/d	0.98(0.15)	1.15(0.22)	1.09(0.34)	1.16(0.26)	1.22(0.36)*
cDPI g/kg/d	0.83(0.21)	0.95(0.23)	0.81(0.26)	0.48(0.63)	0.33(0.34)
nPCR	0.75(0.1)	0.85(0.16)	0.73(0.19)	0.84(0.18)	0.91(0.19)*
Transferrin	1.87(0.23)	2(0.34)	1.79(0.25)	1.6(0.39)	1.6(0.31)
Albumin g/dl	35.1(4.7)	34(34)	32.3(4.5)	34.2(5.3)	36.1(4.3)
Prealbumin	33.2(6.6)	42.8(8)	37.2(8.9)	32.4(10.3)	31(8.8)
IGF-1 iu/l	22.9(8.8)	30.8(14.5)	18.4(8.5)	19(5.9)	17.7(7.9)
Sum skin folds (mm)	55.2(20.1)	47.8(14.6)	63.3(21.5)	48.6(17.9)	43(14)

Figure 15 Those on HD > 1 yr had significantly higher (*p<0.05) FFM, weighed dietary protein intake and nPCR when compared with CrCl<12ml/min/1.73m²

Chapter 3

Relative blood volume measurements during Haemodialysis : Pilot studies

Introduction

Cardiovascular instability with symptomatic hypotension is still one of the major and most frequent complications of dialysis therapy. This limits safe and adequate removal of fluid during HD. Hypovolaemia has been implicated as a major causal factor of morbidity during haemodialysis (HD). Unfortunately the morbidity associated with hypovolaemia is not limited to hypotension. Steuer *et al.* [206] have recently demonstrated that cramping and light-headedness occurred in 28% of all treatment sessions, and in all cases those symptoms were preceded by a pronounced reduction in blood volume (BV). The pathogenesis of intradialytic hypotension remains complex and multifactorial. A common final pathway however is reduction in circulating blood volume [207].

Relative blood volume monitor measures the percentage blood volume change by detecting a change in plasma density and haematocrit during haemodialysis. The initial blood volume is assumed as 100%. The measured plasma density and the corresponding blood volume are recorded continuously and the data stored via data acquisition software along with other machine variables. The change in blood density or haematocrit is inversely proportional to the blood volume changes [208]. Pattern display of such high resolution serial measurements may provide the basis for further detailed analysis of the changes in relation to ultrafiltration and dialysis.

The depicted blood volume profile provides a real time view of the vascular space and allows focus on vascular dynamics. Blood volume monitoring can be used for tracking relative changes in blood volume change during ultrafiltration. Such a changing blood volume profile can be displayed online and stored in memory during the dialytic process. This chapter presents selected pilot case studies, which illustrate a range of relative blood volume (RBV) changes that can be observed during dialysis with or without ultrafiltration.

Relative blood volume changes during Haemodialysis in the absence of ultrafiltration

During initial pilot studies in 5 subjects, I observed that under stable conditions the relative blood volume profile during high flux haemodialysis without ultrafiltration is an almost flat trace with minor fluctuations that may vary approximately between 0.5-1% (Fig 1). Connection of the patient to the extracorporeal circuit using a straight connection the isovolemic stability was within a maximum variation of 1.5 % with a tendency to fall. The bleed out connection on the other hand produced slightly larger variations (within 3.5%). It is also feasible that obligatory ultrafiltration during dialysis without volumetric control machines could produce such variations in blood volume profile in the absence of prescribed ultrafiltration. Major postural changes (sitting or standing from supine), submaximal exercise [209] or hypertonic solutions [210] are other manoeuvres that can significantly influence RBV variation during dialysis without ultrafiltration. A study evaluating the effects of diffusive dialysis on the changes in relative blood volume (RBV) on six stable haemodialysis patients, without the need of ultrafiltration, during 10 sessions of diffusive dialysis (bicarbonate) lasting 4 h with RBV monitoring continuously by measurement of haematocrit showed that during the 1st and 2nd h RBV increased by 2.4 ± 1.4 and $2.5 \pm 0.8\%$ respectively, returning to baseline levels at the end of dialysis. No changes in blood pressure or heart rate were noted. They concluded that during diffusive dialysis without ultrafiltration RBV is increased. A decrease in vascular resistance, or changes in regional blood distribution could also explain these findings. It is also important to bear in mind that haematocrit changes may be more susceptible to osmolarity changes induced by diffusive sodium gradient. This effect is minimal in blood volume monitoring using the ultrasonic method. [211] Achieving stability of relative blood volume trace is essential before other volume dependent alterations to the circulation are examined during dialysis.

Relative blood volume measurement during Haemodialysis with Ultrafiltration

A typical blood volume profile in a single patient during a standard dialysis session with continuous ultrafiltration at a fixed rate is depicted in Fig 2a. The application of ultrafiltration during dialysis has the most significant effect on the relative blood volume profile. There is an almost instantaneous decay of the blood volume curve that corresponds to a simultaneous rise in plasma or blood density and haematocrit. The percentage reduction in blood volume from the baseline 100% starting value, reflects fluid removed by ultrafiltration provided there are no intravenous fluid or drug infusions and there is no evidence of blood loss or red cell destruction within the subject or in the extracorporeal circuit. The decrease in blood volume follows a gradual fall in systolic and mean blood pressure. In the later stages of dialysis when ultrafiltration pump is stopped either due to achievement of dry weight or due to hypovolemic symptoms like cramps or hypotension, the blood volume profile trend is reversed and the profile depicts a fall in plasma density and haematocrit and a simultaneous increase in relative blood volume. The infusion of fluids also has an instantaneous effect on profile with the usual appearance of a sharp spike.

The patient depicted in Fig 2a develops symptoms of cramps and hypotension after 165 mins on dialysis at 92% blood volume at which point the UF was stopped. A steep fall in decay curve corresponds to the haemodynamic instability. Cessation of UF reverses the BV decay allowing for recovery of blood volume and stability. The patient has an effective reduction of 8 % blood volume at the end of dialysis. Fig 2b shows saline induced instantaneous blood volume recovery in a subject following hypotension.

The RBV profiles during standard UF show varying trends within each individual and between subjects. Pilot studies on 12 subjects showed wide RBV variation from 78% to 103%, mean $92.6\% \pm 5.4\%$. Stability of initial blood volume during a period of isovolemia was typically complete within the first 15-20 mins with a variation ranging from 0.1 – 3.7 % mean 1.2 ± 0.63 . Reduction in RBV varies between 6 – 19 %, which is not patient specific. During final refill period between end of UF and end

of dialysis blood volume recovery was typically complete within the first 20 mins with a plateau in 75 % patients with a blood volume stability 0 – 1.5 %, mean 0.35 ± 0.42 . No correlation was found between UF and change in RBV.

In general three broad categories of curves can be described a) flat profile where the blood volume variation was < 3 % b) stable profile with a curvilinear decay with tendency to plateau with progressive flattening c) unstable profile where the blood volume reduction is steep and almost linear. The profile a indicates that the effect of fluid removal had little effect on blood volume reduction and that the patient was grossly overloaded post ultrafiltration. This is the most useful application in day-to-day clinical practice, allowing identification of grossly overestimated dry weight. However the degree of overestimation cannot be accurately judged by such profiles. The profiles b and c are more difficult to categorise, diagnosis often retrospective and subjective in its interpretation.

Non-standard UF prescription can also have a significant effect on the RBV profile. Application of a continuously varying UF rate in a linear decreasing manner and its matching RBV profile is shown in Fig 3. It demonstrates a curvilinear profile with the steeper decay of blood volume at the first hour of dialysis. The patient remains haemodynamically stable throughout despite 4 litres of fluid removal suggesting that variable UF rates with different alternative profiles may be more physiological and allow adequate fluid removal in a safe manner.

APPLICATION OF INTERMITTENT ULTRAFILTRATION PROFILE

The principle of perturbation analysis was applied using short pulses of UF at a fixed rate with intervening rest periods. This allowed study of RBV changes during a UF phase and separately during an isolated refill phase. The method of variable ultrafiltration rate or a sudden stop in UF pump has been used to measure vascular refilling capacity (Schneditz et al) [212, 213]. The use of such a profile can demonstrate vascular refill from fluid filled interstitium during the UF free period evidenced by an increase in the relative blood volume.

Fig 4a and 4b depicts typical intermittent UF profile where I applied bolus sequences that have been gradually reduced in strength (40 – 30 – 20 – 10 % of the total UF volume at high UF rates). This may allow removal of fluid in a graded manner (intermittent linear decreasing rate) despite high UF rates and also provide information on intervening UF free refill periods.

Each UF pulse can be individually analysed in two phases (Fig 5). Two distinct phases are a) period of ultrafiltration depicted by an exponential decay or slope b) subsequent period of no ultrafiltration (refill) depicted by an RBV rise to maximum. Each refill curve pattern seems to be widely variable. Broadly they can be described as an exponential to a maximum rise to a plateau, linear with a plateau or sine wave polynomial. (Fig 6) The variability in the refill curves is more pronounced in the later stages of ultrafiltration. Sometimes the waveform may have more than one definition with a tendency of one waveform to change its configuration and merge into the other waveform.

Conclusion

There is an insignificant change in the blood volume profile during a supine dialysis session with no ultrafiltration. Establishing a stable RBV baseline during isovolemia more easily achieved by a straight connection to the extracorporeal circuit. A period of approximately 20 mins is necessary without ultrafiltration, until a 100% baseline is achieved with accuracy. The application of UF significantly alters the RBV profile during dialysis. Pattern recognition of such changes yield variable curves primarily influenced by UF prescription and wide interpatient and inpatient variability. Steeper blood volume curve more frequently associated with haemodynamic instability. A short single pulse of UF can induce a typical blood volume profile. Characteristic sharp RBV decay is followed by RBV rise to a plateau during the UF free period. Analysis of such UF pulses may provide information on haemodynamic response to UF and dry weight. There is a lack of significant decrease in RBV observed in states of overhydration, while the steepest RBV slope is related to hypovolaemia. Thus, continuous monitoring of RBV during HD could be useful in hypotension-prone patients. Further studies were performed to define the character of the RBV profile during ultrafiltration.

Figure 1 RBV profile during standard dialysis but no ultrafiltration
 Position supine ; UF Rate 0 ml/hr Predialysis wt 65.7 kg DW 66 kg
 Urine output 1.2 litres / 24 hrs

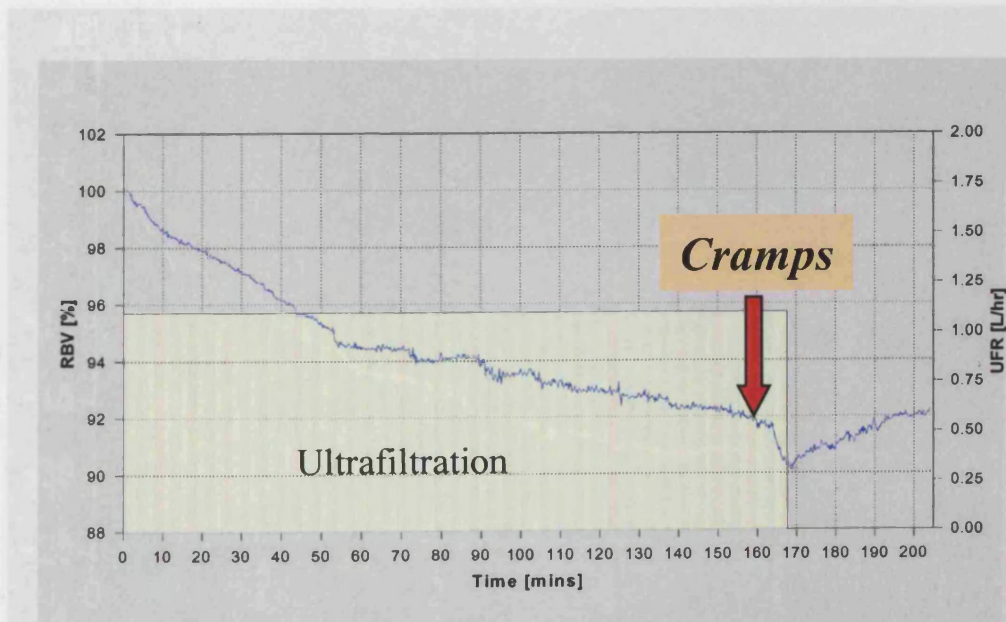
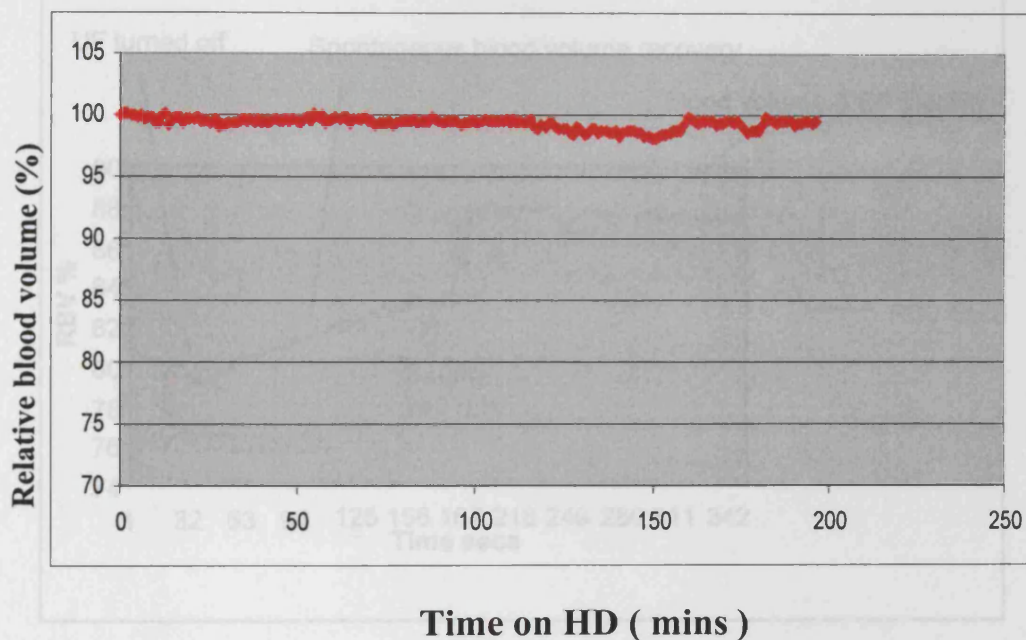


Figure 2a RBV profile during standard continuous fixed UF
 Position supine Predialysis wt 73.2kg DW 70 kg
 UF Rate 1150 ml/hr UFV 3220 ml Delta RBV - 8 %
 Cramps and hypotension at RBV -10%

Figure 2b Instantaneous blood volume recovery and stability with saline bolus

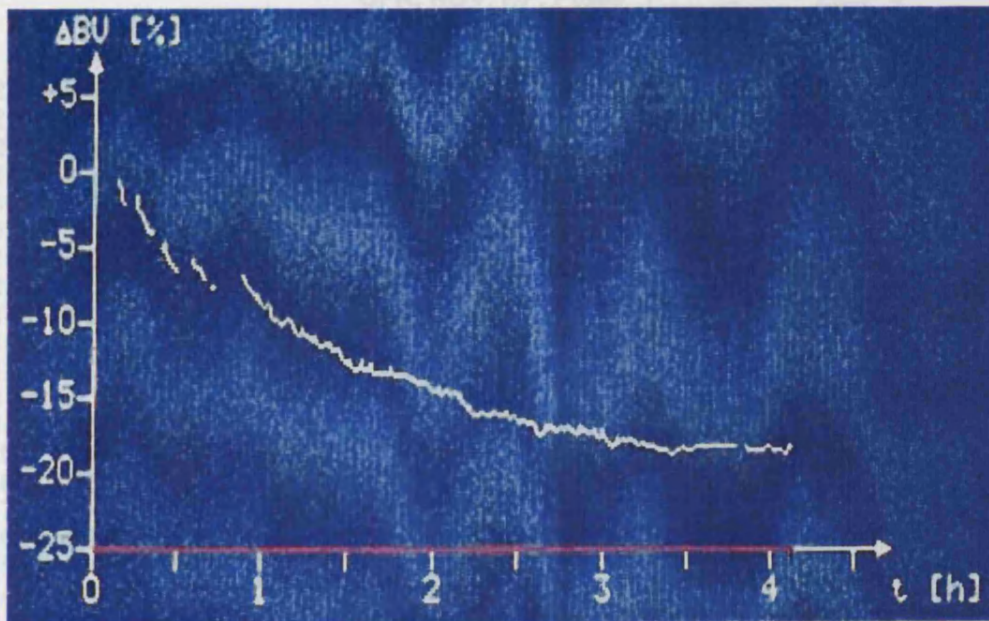
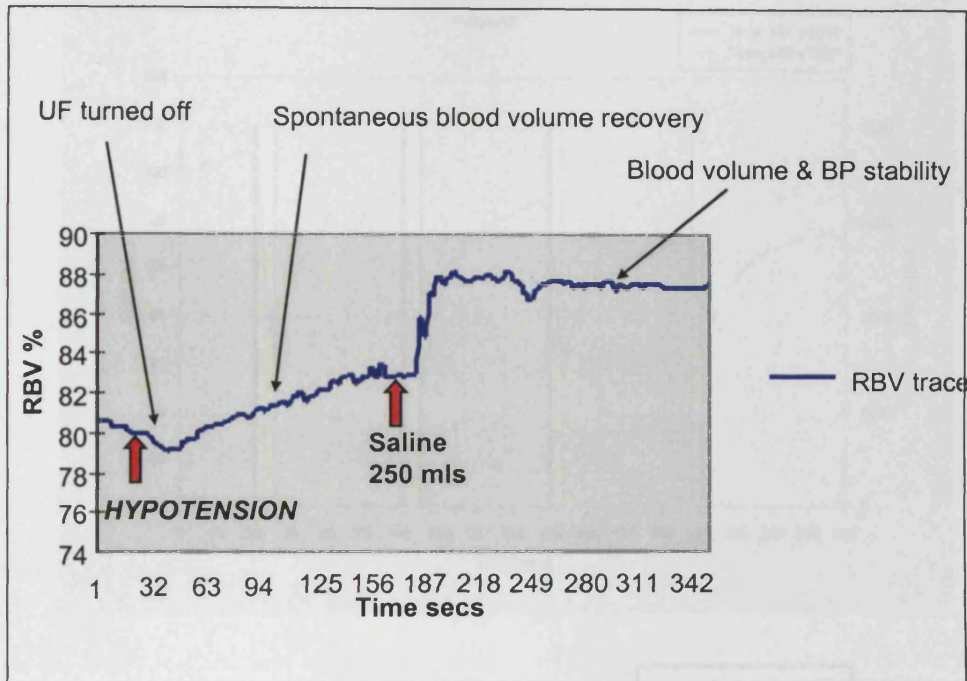


Figure 3 RBV profile with a linear decreasing UF rate
 Supine Predialysis wt 80.1kg DW 76kg
 UF Rate 1800 ml/hr to 170 ml/hr UFV 4000 ml
 Delta RBV - 17.8 % Asymptomatic

Figure 4a Intermittent Ultrafiltration profile (Step UF)

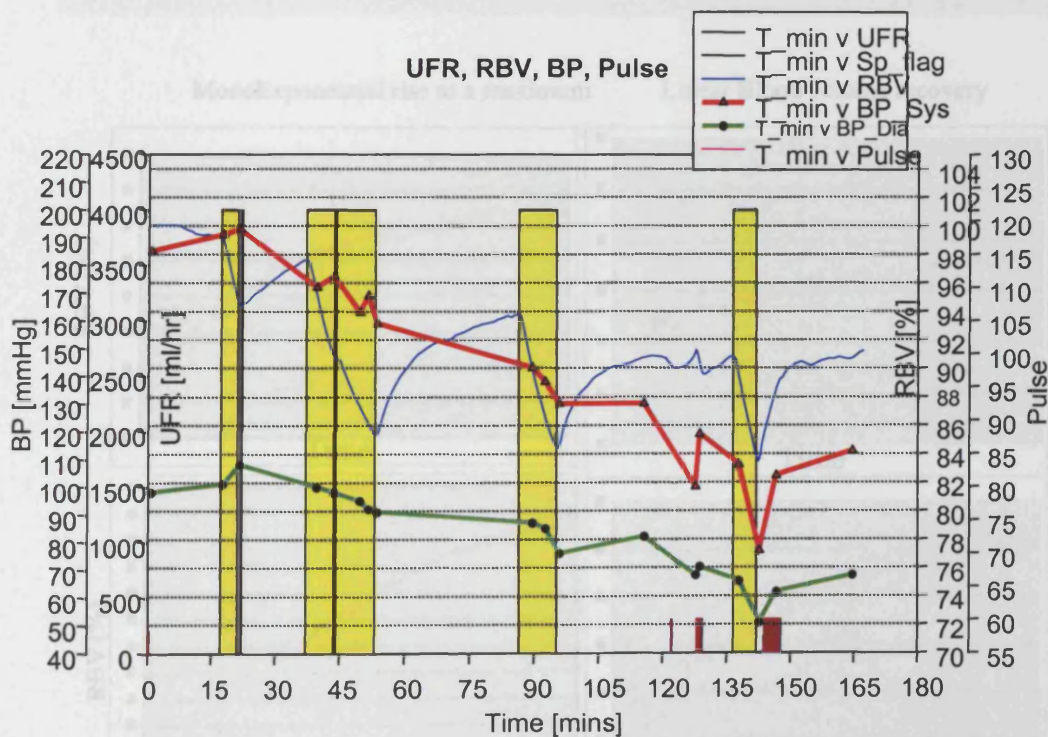
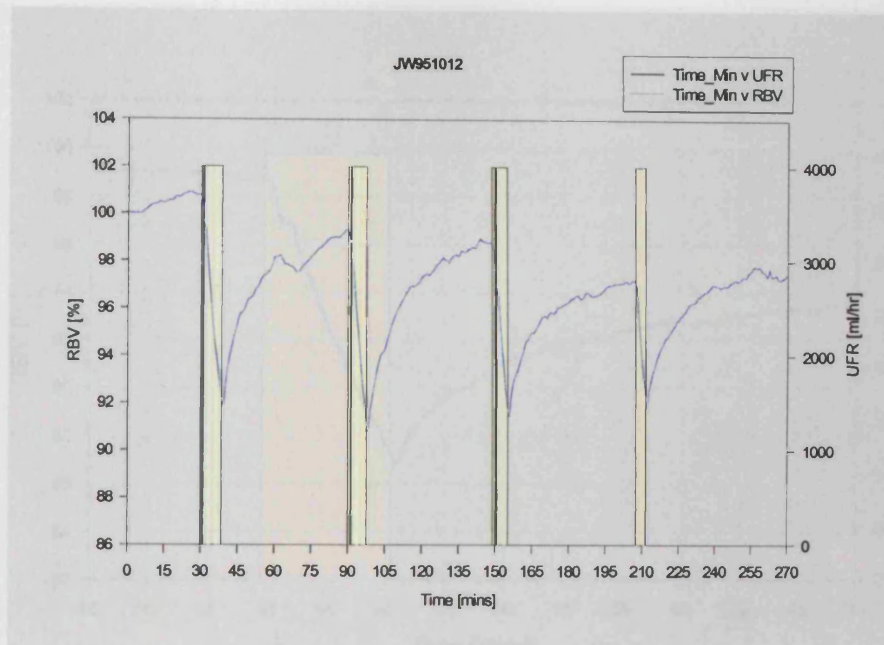


Figure 4b Volume dependent BP reduction during dialysis with step UF

Figure 5 Blood volume response to a single ultrafiltration pulse

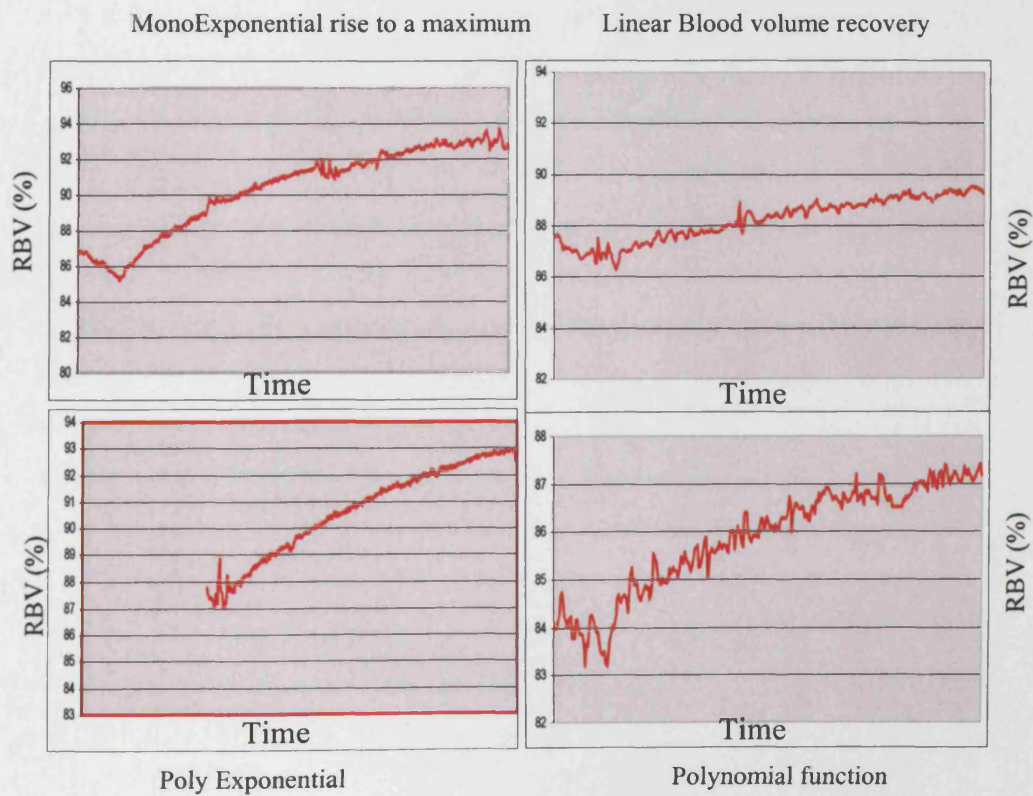
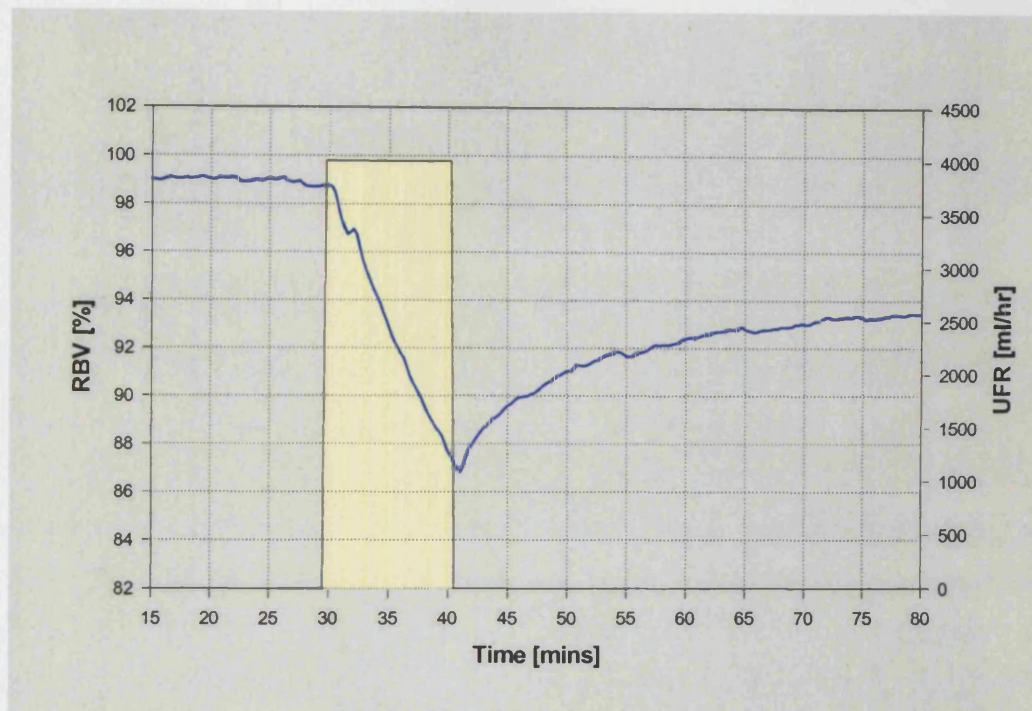


Figure 6 Post UF refill period showing variable response curves

Chapter 4

Characterising Relative blood volume changes during reduction of postdialytic weight in successive dialysis using intermittent UF

Summary

The aim of this experiment was to investigate the relative blood volume curve responses to intermittent step ultrafiltration (UFV_s) at different states of hydration. 12 haemodialysis patients were studied over 40 dialysis treatments in undergoing reduction in their dry weight using an intermittent high rate ultrafiltration profile to observe differences in blood volume responses and vascular refilling at varying degrees of hydration and to try to determine whether these changes can be used to predict the onset of haemodynamic instability during haemodialysis as the dry weight is approached. RBV magnitude (ΔRBV_{UF}), rate of RBV refill (IRR), rbv decay and refill curve coefficients (τ_{UF} , τ_{REF}) were compared at various stages of UF and proximity to dry weight (PDW). ΔRBV_{UF} was significantly correlated with UFV_s ($r=0.81$, $p<0.001$). This applied for all the boli (Step1 $r=0.80$ $p<0.001$; step 4 $r=0.6$, $p<0.01$) By stepwise regression analysis, determinants of ΔRBV_{UF} were UFV_s, IRR, τ_{UF} and patient specific factors ($p<0.01$, $R=0.915$). Factors determining τ_{UF} were $\Delta RBV_{UF}/UFV_s$, hypotension, proximity to PDW ($R^2=0.48$ $p<0.01$) but τ_{REF} was patient specific only ($p<0.001$). Hypotension was predicted by $\Delta RBV_{UF}/UFV_s$ ($p<0.001$), UFVs and τ_{UF} ($p<0.01$) ($R=0.56$, $R^2=0.32$). $\Delta RBV_{UF}/UFV_s$, IRR and τ_{UF} were significantly higher in hypotensive ($n=13$) than nonhypotensive ($n=146$) UF steps ($p<0.01$) even when the analysis was restricted to UFV_s < 250 mls. ($N=13$ Vs $n=49$). In the same patient, $\Delta RBV_{UF}/UFV_s$, τ_{UF} and IRR were significantly different with identical UFV_s between hydration states differing by >1 kg ($p<0.01$). Proximity to PDW at each UF step was predicted by $\Delta RBV_{UF}/UFV_s$, τ_{UF} , IRR ($p<0.01$) and ΔRBV_{UF} ($p<0.05$) ($R=0.63$, $R^2=0.39$). The parameters derived during these studies were strongly dependent on the size of the administered UF bolus.

Introduction

Despite advances in dialysis techniques, determination of dry weight (PDW) and prevention of haemodynamic instability on dialysis remains rather subjective, crude and empirical. Dialysis treatment times have decreased worldwide in recent years, whilst interdialytic weight gains have remained unchanged. High UF rates are therefore required and often varied to achieve the desired fluid removal in the allotted time. This increases the likelihood of an imbalance between UF and vascular refilling. The importance of preservation of the vascular space to a patients' well being during haemodialysis (HD) is well known [214]. To tackle this problem, relative blood volume (RBV) monitoring during UF has been advocated. Hypotension during HD, although multifactorial is largely related to plasma volume depletion. [214, 215] It has been suggested that monitoring relative blood volume changes during UF may allow achieving the prescribed dry weight in a safe manner. [207] The use of technically simple and reproducible tools to characterize such blood volume changes during UF in a refined and more predictive manner may be potentially an invaluable aid to the clinician. There is little data comparing blood volume and vascular refill responses to UF in the same subject at different states of hydration. [216] The use of blood volume changes and intermittent graded high rate UF allows such analysis.

The aim of the study was to define parameters that characterise the relative blood volume changes during ultrafiltration at varying states of hydration and determine whether blood volume profile during UF may predict incipient hypotension and proximity to dry weight. The principle of perturbation analysis was applied by using short bursts of UF at a high rate with intervening rest periods. This allowed study of RBV changes during a UF phase and separately during an isolated refill phase.

EXPERIMENTAL SET UP 1

Patients and Methods

Patient Characteristics

Stable patients on maintenance HD with adequate haemoglobin (>10 g/dl) and serum albumin (> 30 g/dl) levels were enrolled into the study. Haemodynamically unstable patients (those with cardiac failure and those with frequent hypotension during HD) and those with an unstable vascular access were excluded. The patients had been on HD for a mean 16 ± 5 months and the mean age was 58.5 yr. (range 25-76 yrs). The mean haemoglobin and serum albumin levels at start of the study were 11.1 ± 0.5 g/dl and 37 ± 2 g/dl respectively. Informed consent was obtained from all subjects. The North Herts Ethical Review Committee approved the study.

Dialysis technique

All patients received high flux HD 3 times per week using bicarbonate buffer, $1.0\text{--}1.4$ m² hollow fibre polysulfone membranes with blood flow rates of 300-500ml/min, dialysate flow rates 800 ml/min and the Fresenius delivery system with an inbuilt ultrasonic blood volume monitor. Dialysis was prescribed according to a UKM [112]. Dialysis fluid composition according to standard specifications in the unit (see Methods) with the dialysate temperature maintained at 36 degrees Centigrade. Patients had previously been ultrafiltered to their clinically defined dry weight. At high UF rates the transmembrane pressure across dialyser reached high levels but remained below the safety limit given by the manufacturers. No food or drink, were allowed during dialysis sessions. Mean ultrafiltration (UF) volume (UFV) ranged from 900 - 3500 mls and the mean treatment time was 3hr 30 mins.

Blood volume monitoring

Blood volume (BV) monitoring was performed using real time online ultrasonic blood volume monitor (Fresenius BVM), which measures the velocity of sound across flowing blood (using a cuvette in the extracorporeal circuit designed for this purpose) depending on changes in the density of total protein content (sum of plasma proteins and haemoglobin). The relative blood volume (RBV) at any given point of

time may be determined from the changes in the protein concentration relative to the initial starting value.

Definitions

Dry weight:

Dry weight was defined as the lowest weight a patient could tolerate without the development of hypotension or suggestive symptoms such as dizziness, faintness, nausea or cramps

Hypotension

Hypotension was defined as a systolic BP < 90 mm Hg or a fall in mean BP of > 30 mmHg associated with symptoms of suggestive of hypovolemia, such as dizziness, faintness, nausea or cramps.

Protocol

Variable volume step ultrafiltration

Forty treatments were initially monitored in 12 patients who were undergoing a sequential reduction of their dry weight over successive dialysis. An identical UF profile was used in all treatments. Following an isovolemic period in supine position (20 min equilibration phase), intermittent UF pulses were applied sequentially in order to remove 40%, 30%, 20% and 10% of the total UF volume for that session (in the same order) at a rate of 3l/hr. Each UF step was followed by an UF free phase of 20-30 minutes to allow equilibration. Fig 1 The UF step volume (UFV) ranged from 100 – 1050 ml. The post dialytic weight was reduced by 0.5 kg at each HD during successive treatments. The occurrence of a hypotensive episode near the end of the treatment was the defined endpoint. A symptomatic dialysis session was defined as one in which hypotension occurred necessitating cessation of UF or infusion of saline. No food or drink was allowed during dialysis.

Definition of Parameters

ΔRBV_{UF} (%)	The magnitude of RBV change during UF
$\Delta RBV_{UF}/UFV_s$ (%/ml)	The magnitude of RBV change during UF per ml of UF volume
UFV_s (ml)	Volume of ultrafiltration pulse
ΔRBV_{ref} (%)	The magnitude of RBV change during the refill period following the UF pulse
τ_{UF}	Decay constant for a single exponential decay
τ_{ref}	Constant for an exponential rise to a plateau
IRR (%/min)	Initial refill rate-the refill volume during the first minute after stopping UF

Derived Parameters. i. Decay constants. Automated curve fitting analysis was used to analyse the best-fit equation ($n = 8500$ Adjusted fit $r^2=0.98$, least squares regression analysis) using Table curve 2D software (see methods). In order to determine the decay constants the RBV responses were fitted into single exponential functions:

$$y = b e^{-x/c} \text{ for the UF phase, and [168]}$$

$$y = a + b [1 - e^{-x/c}] \text{ for refill phase. [168]}$$

The major parameters derived Fig 1 was, in the UF phase, the **ultrafiltration decay constant** c (τ_{UF}), and in the refill phase, the **refill phase constant** c (τ_{ref}). Other parameters were the value 'b' in the above equations, during UF representing the amplitude of RBV changes (**UF decay amplitude**) and during refill representing the amplitude of refill RBV change (**refill phase amplitude**) with the refill starting at point 'a'. The fitting was done using 100 iterations. Only constants derived from curve fits with dependencies > 0.80 was used for analysis.

Statistics The statistical tests used were the Students' t-test (paired) for differences between means and Pearson correlation coefficient. Chi-squared analysis was used for unpaired data. All analyses were done using the SSPS statistical package and Sigma plot statistical software (Jandel Scientific) [see methods]

Results

1. Fixed UFR , Variable volume step UF (40 Treatments)

The mean RBV reduction during a hypotensive dialysis session $14.1 \pm 4.7 \%$ was significantly higher than other nonhypotensive dialyses ($10 \pm 3.6 \%$). Fig 2 depicts a typical RBV profile with intermittent uf in a subject enrolled at the start of the study. Fig 3 in the same patient depicts the RBV profile at the final hypotensive dialysis session. Significant reduction in BP, RBV and postdialysis occurred in the hypotensive sessions. (Table 1) Mean reduction in PDW per patient over the study was 1960 ± 650 mls. ΔRBV_{UF} was significantly correlated with UFV_s ($r=0.81$, $p<0.001$). This applied for all UF pulses (Step1 $r=0.80$ $p<0.001$; step 4 $r=0.6$, $p<0.01$) The measured blood volume decay parameters and rate of change in blood volume during UF and refill phase at 1, 5 and 10 mins are depicted in Table 2 and Table 3.

Fitting experimental data.

Mean dependency was $0.89 \pm 0.035(b)$, and the coeff of variation 1.23%. Unlike the exponential fit for UF the fitting of refill data was less satisfactory. [168]

1.1 Determinants of change in relative blood volume

The change in RBV during a pulse of ultrafiltration (ΔRBV_{UF}) was highly correlated with the volume of that UF pulse (UFV_s) ($r=0.84$, $p<0.001$). Fig 4 The rate of change in blood volume in the first and second UF pulses was significantly correlated with the volume of the ultrafiltrate (boli1 $r=0.90$, $p<0.01$; boli2 $r=0.89$, $p<0.01$). This correlation was also observed in the later uf pulses (prefinal pulse $r=0.80$, $p<0.01$; final pulse $r=0.42$ $p<0.05$) although at lower significance. (Fig 5)

By stepwise multiple regression analysis ($R^2=0.743$) the determinants of ΔRBV_{UF} were UFV_s ($p<0.001$), patient specific factors ($p=0.015$), and distance from dry weight ($p<0.041$). (Fig 6) Whether or not hypotension occurred during the bolus was not a significant determinant. When ΔRBV_{UF} during a bolus was corrected for bolus

size ($\Delta RBV_{UF}/UFV_s$), whether or not hypotension occurred during the bolus became a significant determinant ($p<0.001$) but in this model, distance from dry weight was not a significant factor. UFV_s remained a highly significant determinant ($p<0.001$) (overall $R^2=0.239$). The only significant determinants of ΔRBV_{ref} (overall $R^2=0.336$) were the bolus number and UFV_s (both $P<0.001$). The predictors of IRR (overall $R^2=0.239$) were the bolus number and UFV_s (both $P<0.001$), and proximity to dry weight ($p=0.003$).

1.2 Determinants of ultrafiltration decay constant (τ_{UF}) and refill constant (τ_{REF})

Factors determining τ_{UF} were whether or not hypotension occurred during the bolus ($p<0.001$), and proximity to dry weight ($p=0.02$) (overall $R^2=0.222$) but τ_{REF} was patient specific only ($p<0.001$) (overall $R^2 = 0.113$) (Fig 6)

1.3 Predictors of hypotension and proximity to dry weight

Proximity to dry weight at each UF step correlated with ΔRBV_{UF} ($R=0.50$; $p<0.001$), UFV_s ($R=0.5$; $p<0.001$) and τ_{UF} ($R=-0.36$; $p=0.064$). By multiple regression analysis ΔRBV_{UF} and τ_{UF} emerged as the significant predictors of proximity to dry weight (overall $R^2=0.35$ and $p<0.001$ in both cases). In logistic regression analysis, hypotension was predicted by ΔRBV_{UF} ($p=0.015$) and $\Delta RBV_{UF}/UFV_s$ ($p<0.001$) (Fig 6).

$\Delta RBV_{UF}/UFV_s$ (2.64 ± 0.65 vs 1.72 ± 0.65), and τ_{UF} (21.3 ± 4.4 vs 14.4 ± 14.2) were significantly higher and UFV_s (149 ± 53 vs 362 ± 199) and ΔRBV_{UF} (3.80 ± 1.35 vs 5.91 ± 3.07) were significantly lower ($p<0.001$ in all cases) in hypotensive ($n=14$) compared with non-hypotensive ($n=146$) UF steps.

Initial refill rate (IRR)

The initial refilling rate (calculated immediately after a period of ultrafiltration) was limited to a mean 0.78 ± 0.13 %/min (Fig 7a). At very low blood volumes (RBV reduction of more than 15%) this may be exceeded (Fig 7b).

Discussion

This experiment investigates the relationships between relative blood volume responses, vascular stability and dry weight during intermittent ultrafiltration. The blood volume decay characteristics were overwhelmingly influenced by the UF volume and rate. However the UF decay constant and the rate of change in blood volume predicted hypotension and proximity to dry weight when adjusted for the ultrafiltrate volume.

The lack of strong correlation of simple RBV parameters to approaching dry weight (ΔRBV_{UF} barely reaching significance at $p < 0.04$) could be explained by various factors. Susceptibility to hypotension under conditions of hypovolemia is largely dependent on the performance of the patients' compensatory mechanisms. Apart from the overriding influence of UF volume, vasoactive medications, vascular compliance, sympathetic responses and cardiac contractility are perhaps significant contributory factors. The absence of ultrafiltration during the refill phase allows the other factors to become dominant in shaping refill responses. Consequently blood volume changes during refill phase are highly patient specific. A further consideration would be the fact that excess blood volume and interstitial fluid are not linearly distributed due to Guyton relationship.[78]

Following a period of ultrafiltration there is a refilling phase as fluid from interstitial space replenishes the vascular compartment compensating for blood volume loss. Refilling time and rates during periods of no ultrafiltration was examined to analyse vascular refilling. This was done using data from a selection of arbitrary time points during refilling process.. The first minute refill post UF cessation correlated with haemodynamic changes, perhaps reflecting an effect of haemodynamic compensation mechanisms on this refill parameter or refill capacity. The initial refill rate seemed to be plateau between RBV 85 – 95% but tended to be higher at very low blood volumes (RBV<85%). This phenomenon (initial forced refill) in conditions of severe plasma volume depletion could be due to underlying physiological processes i.e. vasoconstriction or splanchnic effect. It is also unclear whether recirculatory effects could affect these changes. A few patients prior to developing hypotension are

capable of a higher initial refill rate an important consideration in the design of blood volume control systems.

Parameters derived for refill curve analysis in this experiment disappointingly failed to convincingly predict patients prone to hypotension or with poor refill characteristics. The refill response curve was predominantly patient specific. The rate of refilling is dependent on factors such as the size of the vascular space, the degree of hydration, oncotic forces and the performance of active refill processes etc. Monoexponential fitting of refill phase of the blood volume curves were less also satisfactory than UF phase with lower dependencies. This probably indicates that the refilling phase is an integral function of multiple physiological forces as opposed to UF where the high UF being the dominant physiologic process tends to have a more pronounced effect on the blood volume changes.

RBV reduction adjusted for volume of ultrafiltrate ($\Delta RBV_{UF}/UFVs$) and ultrafiltration decay constant (τ_{UF}) seemed to be the two most powerful predictors of approaching dry weight and impending haemodynamic instability. This perhaps suggest that the hydration status and the haemodynamic effects of ultrafiltration have a major influence on the shape the RBV profile. Linear and nonlinear equations that characterise physiologic processes are defined by their coefficients and basic function (b, $1/c$ (τ_{UF}), cumulative area under curve (AUC)) and not just the appearance of the curve (Fig 8). To investigate this, in the next phase of study intermittent equal steps of UF volume at a fixed rate were applied to investigate the blood volume responses (curve decay characteristics and coefficients) to ultrafiltration at differing states of hydration.

Table1. Comparing hypotensive and nonhypotensive dialysis sessions

Blood volume Parameters	Non-hypotensive		Hypotensive	
	Mean	SD	Mean	SD
ΔRBV_{UF} (%) *	10.0	3.6	14.1	4.7
ΔRBV_{UF} (%) / weight (%/kg) *	.11	.05	.18	.08
Systolic BP reduction (mmHg) *	24	18	52	10
Predialysis body weight (kg) *	92.4	24.1	87.2	26.5
UFV (mls)	1689	734	1674	590

*P<0.05 SD=standard deviation, UFV= ultrafiltration volume, RBV= relative blood volume

Table 2. Blood volume responses to Ultrafiltration pulse

	Pulse 1 (40%)		Pulse 2 (30%)		Pulse 3 (20%)		Pulse 4 (10%)	
	NH	H	NH	H	NH	H	NH	H
Volume (ml)	597(350)	590(190)	485(120)	435(180)	314(172)	329(96)	157(90)	165(42)
Bolus Width (min)	13.6(7.6)	8.9(4.2)	8.9(5.5)	7.5(2.7)	7.1(4.3)	6(1.9)	4.8(2.3)	5(2.8)
ΔRBV_{UF} (%)	9.2 (3.7)	9.5 (2.4)	8.5 (3.1)	7.4 (2.0)	5.8 (2.7)	5.6 (1.4)	3.5 (1.9)	4.7* (1.3)
ΔRBV_{UF} (%) / ΔRBV corrected for BV (%)	7.2 (3.6)	6.5 (2.4)	5.6 (3.5)	5.8 (2.0)	4 (1.2)	4.75 (1.5)	3.1 (1.3)	4.9* (1.5)
ΔRBV_{UF} (%) at 1 min of UF	0.99 (.61)	0.99 (0.44)	1.12 (0.46)	1.1 (0.4)	1 (0.75)	1 (0.4)	1.09 (0.6)	1.0 (0.42)
ΔRBV_{UF} corrected for UF volume (%/l)	14.9 (4)	16.8 (3)	15 (3.8)	17 (3.6)	17.4 (8)	17 (4)	18.6 (1.4)	20* (5.6)

ΔRBV_{UF} = reduction in relative blood volume during UF.

NH= nonhypotensive treatment H= hypotensive treatment

Figures in parentheses indicate sd of mean. * P<0.05 between H and NH treatments

Table 3. Rebound Blood volume (Refill responses) to individual Ultrafiltration pulse

Parameters	Rebound 1		Rebound 2		Rebound 3		Rebound 4	
	NH	H	NH	H	NH	H	NH	H
Duration(mins)	21.8	19	22	21.4	22.9	19	20.4	17.1
ΔRBV_{ref} (%)	3.99 (2.6)	3.43 (1.1)	3.49 (2.11)	3.33 (1.38)	3.44 (2.24)	3.29 (1.08)	2.83 (1.81)	3.08 (1.46)
1 min refill rate (%/min)	0.91 (0.61)	0.77 (0.27)	0.74 (0.46)	0.88 (0.44)	0.95 (0.79)	0.95 (0.35)	0.96 (0.99)	1.1 (0.55)
5 min refill rate (%/min)	0.25 (0.19)	0.07 (0.41)	0.21 (0.23)	0.16 (0.15)	0.13 (0.24)	0.14 (0.21)	0.14 (0.24)	0.22 (0.33)
10 min refill rate (%/min)	0.04 (0.35)	0.14 (0.14)	0.07 (0.20)	0.06 (0.21)	0.05 (0.18)	0.12 (0.13)	0.14 (0.22)	0.04 (0.23)

ΔRBV_{ref} = increase in relative blood volume during refill phase.

NH= nonhypotensive treatment H= hypotensive treatment.

Figures in parentheses indicate sd of mean. No significant differences obtained

Figure 1 Step Ultrafiltration Profile

Blood volume response to an UF bolus followed by a UF free refill phase

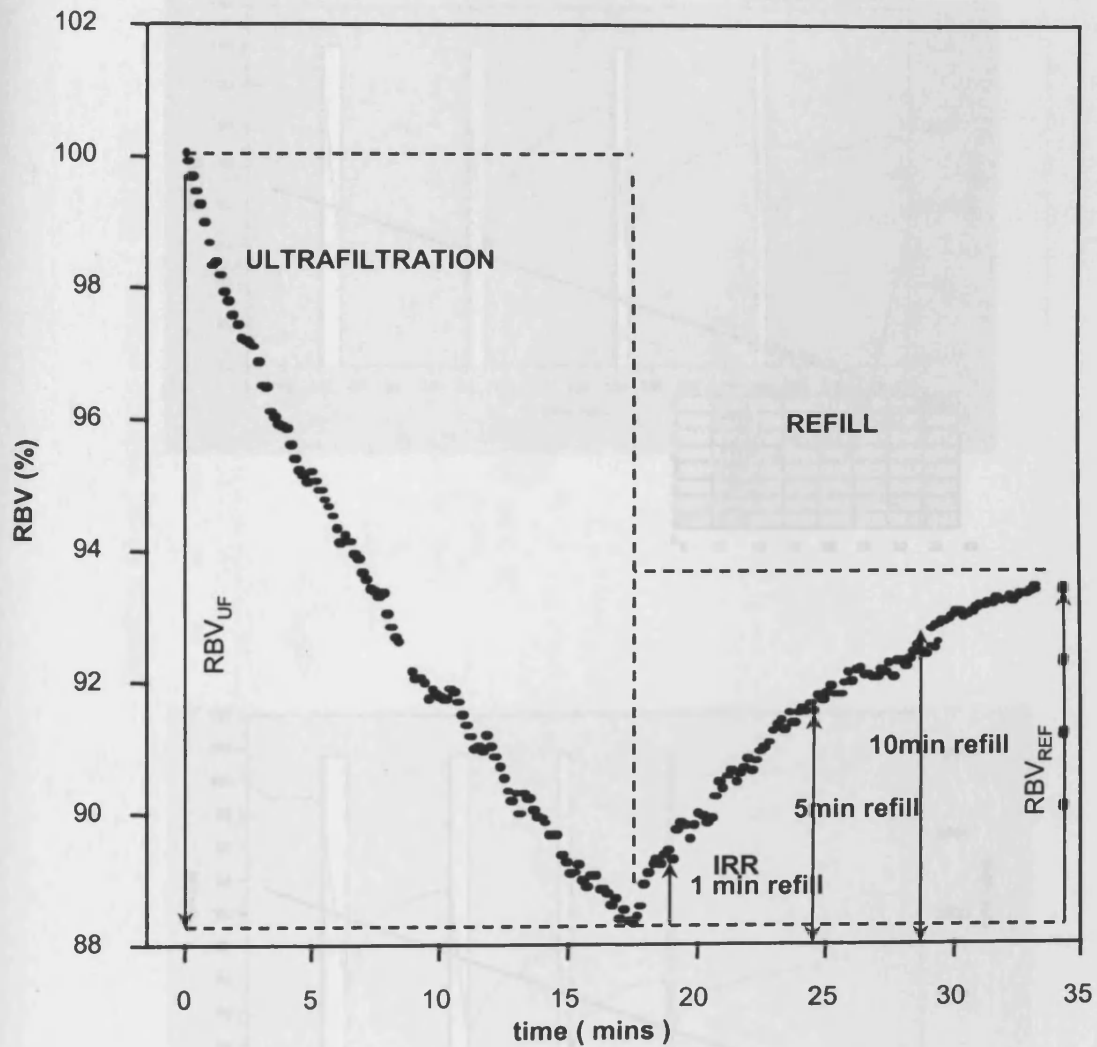


Figure 3 A typical profile in the same subject post weight reduction
Inset : Near normal hydration state superimposed on schematic Guyton curve

Figure 4 Correlation of the volume of the ultrafiltrate step at

Figure 2 A typical Step UF profile in a subject at start of experiment 2

Inset : Overhydration state superimposed on schematic Guyton curve

(n=160)

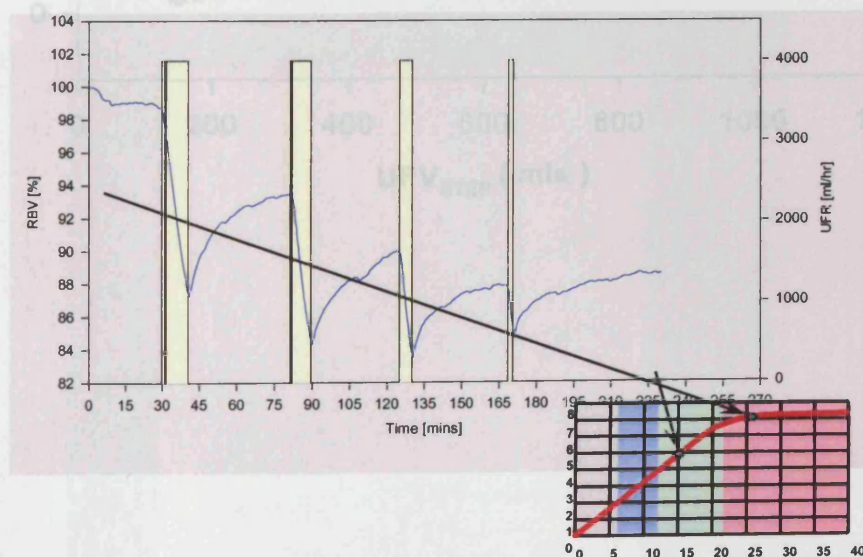
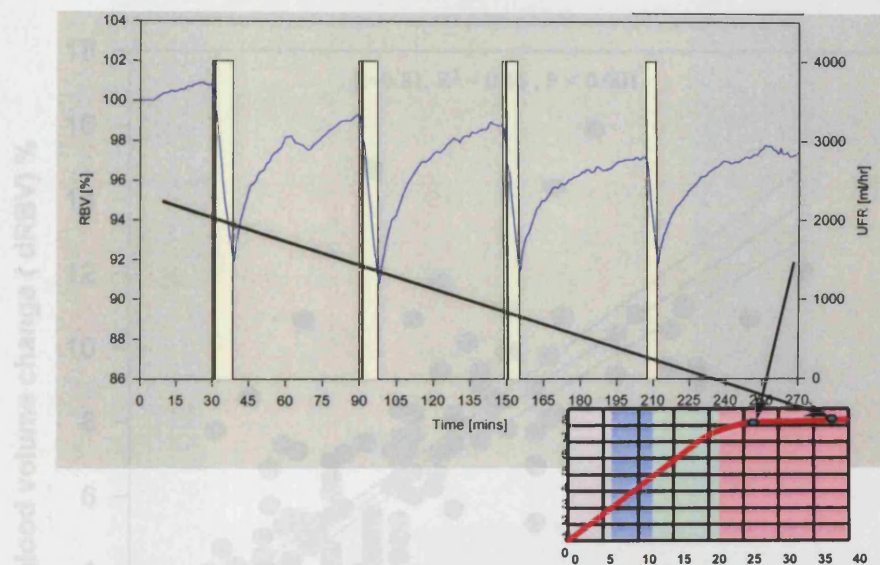


Figure 3 A typical profile in the same subject post weight reduction

Inset : Near normal hydration state superimposed on schematic Guyton curve

Figure 5 Step UF Volume and RBV reduction

Figure 4 Correlation of the volume of the ultrafiltrate step at each bolus and the magnitude of relative blood volume change in Experiment 1 (Regression line with 95% confidence intervals) (n=160)

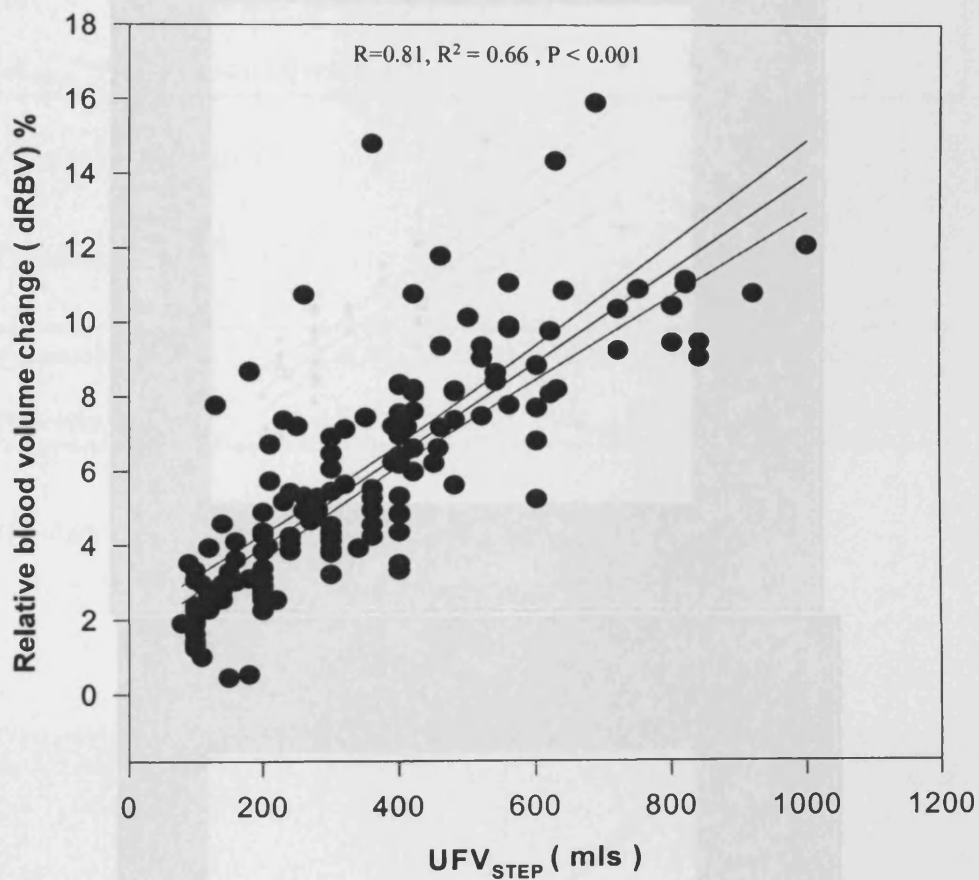


Figure 5 Step UF Volume and RBV reduction (d_RBV_{UF}) demonstrating significant correlation for first and final ultrafiltrate pulse.

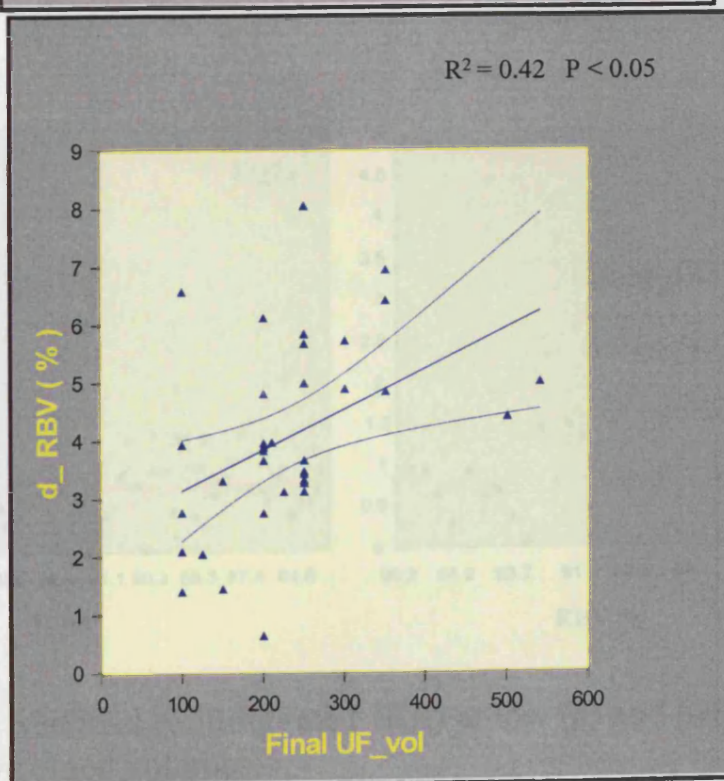
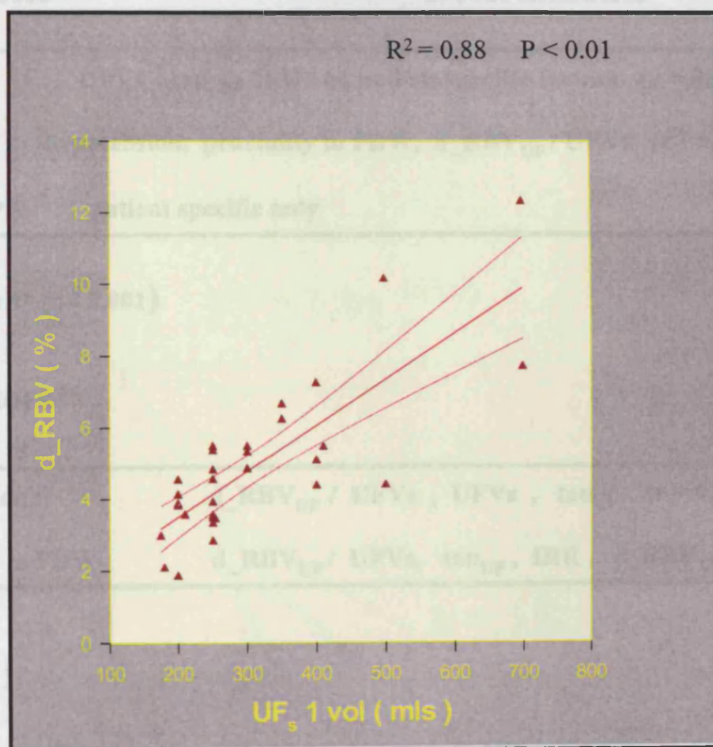


Figure 6 Stepwise regression analysis for determinants of UF and refill characteristics and predictors of hypotension and proximity to dry weight

Variables	Determinants
$d_RBV_{UF}^*$	UFVs , τ_{UF} , IRR and patient specific factors ($r = 0.915$)
τ_{UF}^*	hypotension, proximity to PDW, $d_RBV_{UF} / UFVs$ ($r^2 = .48$)
τ_{REF}^{**}	patient specific only

* ($p < 0.01$) ** ($p < 0.001$)

Predictors*

Hypotension	$d_RBV_{UF} / UFVs$, UFVs , τ_{UF} ($r = 0.56$)
Proximity to PDW	$d_RBV_{UF} / UFVs$, τ_{UF} , IRR , d_RBV_{UF} ($r = 0.63$)

* ($p < 0.05$)

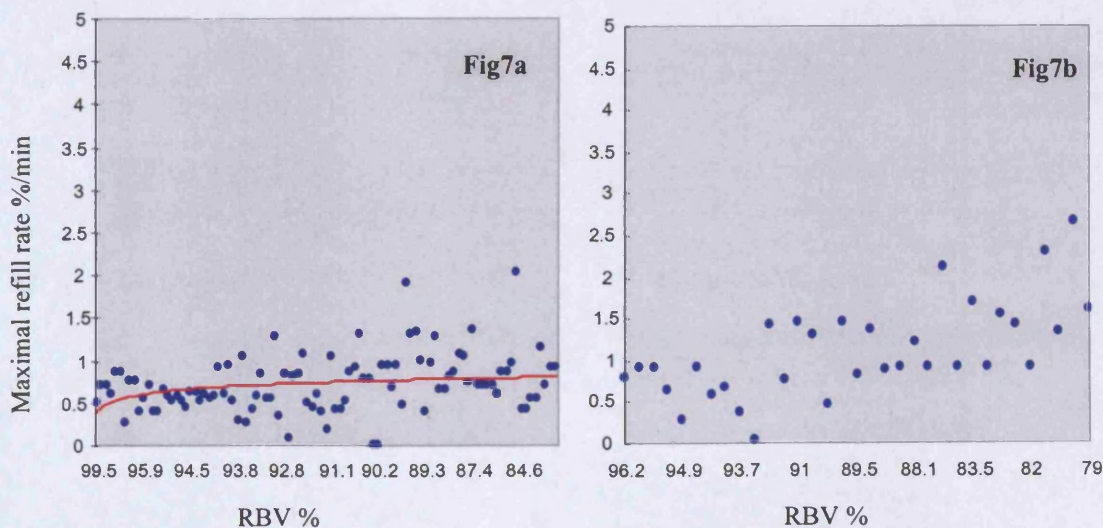
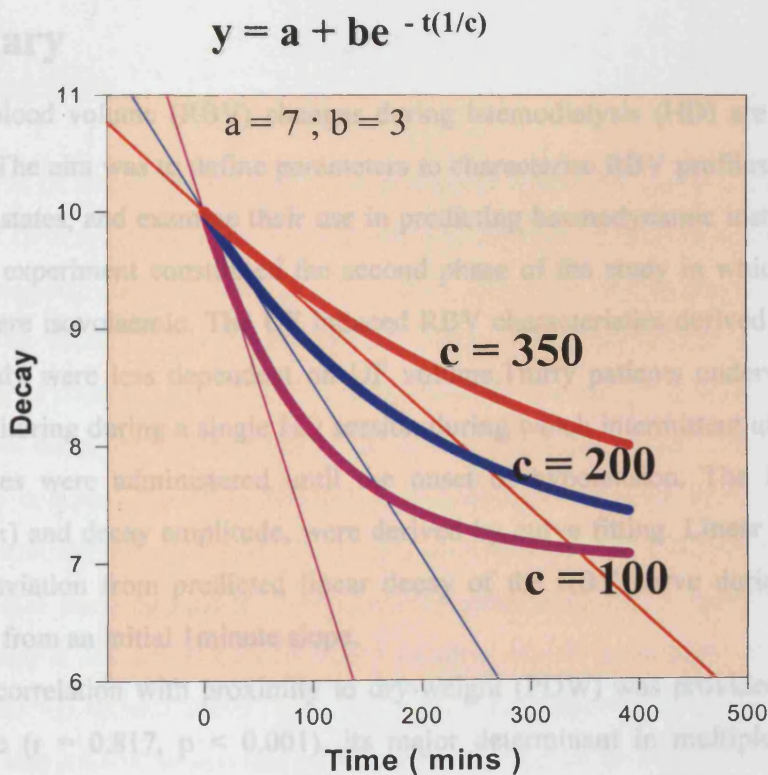


Figure 7 Maximal refilling rate (IRR) at low (a) and extremely low (b) relative blood volumes.

Chapter 5

Figure 8 Simulation of the single exponential [$y = be^{(-x/c)}$] decays (Exp curves A, B, C) with identical coefficient [b (amplitude) = 10] and varying decay constant (c) [c (rate constant) = t (tau)]. The equivalent parameters on a UF induced exponential decay curves are $c = t_{UF}$, and $b = \text{UF decay amplitude}$.



Chapter 5

Analysis of the relative blood volume changes during intermittent isovolemic step ultrafiltration and its relationship to hypotension and proximity to dry weight

Summary

Relative blood volume (RBV) changes during haemodialysis (HD) are difficult to interpret. The aim was to define parameters to characterise RBV profiles at different hydration states, and examine their use in predicting haemodynamic instability. The following experiment constituted the second phase of the study in which UF steps applied were isovolaemic. The UF induced RBV characteristics derived in this part of the study were less dependent on UF volume. Thirty patients underwent online RBV monitoring during a single HD session during which intermittent ultrafiltration (UF) pulses were administered until the onset of hypotension. The RBV decay constant (τ) and decay amplitude, were derived by curve fitting. Linear divergence, the net deviation from predicted linear decay of the RBV curve during UF, was computed from an initial 1 minute slope.

The best correlation with proximity to dry-weight (PDW) was provided by Linear divergence ($r = 0.817$, $p < 0.001$), its major determinant in multiple regression analysis. Other predictors were RBV at initiation of UF pulse, UF pulse volume and UF decay constant (τ_{UF}). These parameters were significantly different in UF pulses within 1kg and ≥ 1 kg of dry weight. There were no correlations with refill parameters. The occurrence of hypotension was not different at RBV $<90\%$ [7.4%] and $\geq 90\%$ [5.3%]. τ_{UF} , Linear divergence, RBV at initiation of UF pulse (all $p < 0.001$), and UF decay amplitude ($p < 0.01$) were different between hypotensive and normotensive UF pulses. Hypotension was the only independent predictor of τ_{UF} ($R^2 = 0.40$; $p < 0.001$). The only independent predictor of Linear divergence was PDW ($R^2 = 0.667$; $p < 0.001$).

Linear divergence and τ_{UF} , measures of the deviation from linearity of the RBV curve during UF, predict hypotension and thus more helpful in predicting the onset

of haemodynamic instability as dry weight was approached. The most useful of these parameters was a quantity we have designated the “refill divergence”, which is a measure of the deviation from linearity of the RBV curve during UF. Approaching dry weight the RBV decline during UF switched from exponential to linear decay, probably indicating failing vascular refill. Monitoring deviation from linearity may allow improved haemodynamic stability and attainment of optimal post-dialysis weight.

Introduction

The process of ultrafiltration (UF) has a marked effect on the hydrostatic forces within the fluid compartments. Initially the ultrafiltered volume is withdrawn from the intravascular compartment. A fluid shift from the overhydrated interstitium towards the intravascular space (refill) forms the predominant compensatory mechanism to overcome or diminish hypovolemia. The rate of vascular refilling is therefore an important determinant of the extent of plasma volume depletion. [217] These two complex processes interact with each other to alter fluid shifts across compartments and the effects of this interaction may vary with differences in volume status. It has been suggested that haemodynamic stability is less likely to be compromised if the proportion of blood volume, which is removed during a HD session does not exceed 8-10% per hour [207]. The fundamental problem with this concept is that it takes no account of the patient's response to fluid removal, whether physiological or pathological. Standardised parameters to define optimal blood volume reduction are well understood. This severely limits its clinical utility.

The aim of this study was to define parameters, which might characterise RBV changes during isovolemic UF pulses at varying states of hydration, and to assess whether using these might help predict haemodynamic instability during UF. The following experiment constituted the second phase of the study in which UF steps applied were equal in volume and rate (isovolemic) during a single dialysis session. The UF induced RBV characteristics derived in this part of the study were therefore less dependent on UF volume.

The most useful of the parameters we derived, was a quantity that I have designated the “linear divergence”, which is a measure of the deviation from linearity of the RBV curve during UF. The use of such technically simple and reproducible tools to characterize RBV changes during UF in a predictive manner may be potentially valuable clinically.

(A) EXPERIMENTAL SET UP 2

Patients and Methods

Patient Characteristics

Thirty stable patients on maintenance HD with adequate haemoglobin (> 10 g/dl) and serum albumin (> 3 g/dl) levels were enrolled into the study. Patients within 3 months of starting dialysis, hemodynamically unstable subjects (those with cardiac failure and frequent hypotension during HD) and those with suboptimal vascular access were excluded. The studied patients had been on HD for a mean 13 ± 3 months and had a mean age of 54.5 yr. (range 22-77yrs). The mean haemoglobin and serum albumin levels at start of the study were 10.8 ± 0.6 g/dl and 3.8 ± 0.3 g/dl respectively. The predialysis blood pressures were 141.8 ± 19.6 (systolic), 75.4 ± 10.9 (diastolic) and 97.5 ± 11.6 (mean) mm Hg. Thirteen subjects were not receiving antihypertensive medications, 13 subjects were taking one agent, 3 were taking two agents and 1 was taking three agents. Informed consent was obtained from all subjects. The North Herts Ethical Review Committee approved the study.

Dialysis technique (see Chapter 4, Experiment 1 & Methods)

Blood volume monitoring (see Chapter 4, Experiment 1 & Methods)

Definitions of Dry weight: & Hypotension (see Chapter 4, Experiment 1)

Protocol

i. Thirty subjects with stable dry weight were studied during a single midweek

dialysis session.

ii. UF during the study was performed as follows: the initial UF pulse removed 40% of the interdialytic weight gain (IDWG) followed by a UF free equilibration period of 20-30 minutes. Subsequently, 3 equal, intermittent UF pulses (20% IDWG) were carried at a rate of 3l/hour (isovolemic pulses for each study) with UF-free equilibration periods of at least 20 minutes between pulses until the patient became hypotensive. If hypotension had not occurred after 3 UF pulses, additional pulses of the same volume were administered until the onset of hypotension.

iii Treatment of hypotension consisted of stopping UF and, if necessary, infusion of saline (approximately 100ml) at repeated intervals till the BP recovered. The post dialytic weight on that day was then set as the achieved dry weight. **The proximity to the dry weight** at any point during the dialysis session was determined retrospectively, as the UF volume (in millilitres), which would need to be removed to reach this achieved dry weight.

iv Blood pressure (BP) and pulse rate was recorded every 20 min or more often when suggestive symptoms occurred.

v. Only data from RBV profiles obtained during isovolemic pulses (i.e. those during which 20% of total ultrafiltered volume was removed) were utilised in subsequent analyses. Data from the initial UF pulse (during which 40% of the UF volume was removed) were not analysed. This is because in pilot studies, UF pulse volume was a major determinant of RBV decay characteristics, tending to obscure the effects of patient specific factors on this parameter. RBV data following saline infusion were censored and excluded from the analysis since the RBV profile may have been altered by the presence of saline in the extracorporeal circuit.

Data Acquisition

A customised data acquisition system using dedicated software (including Microsoft Excel) and a personal computer was used to store and analyse experimental data.

Filtering of data was required prior to precision analysis. The data were processed in two steps.

Step 1 Filter processing: Raw relative blood volume data was processed using a smooth filter function to reduce noise in the data [218]. Reversing the filtered data output in a second pass through the filter eliminated possible distortion due to time delay resulting from the filter's non-linear phase shift.

Step2: RBV transition and RBV transition time. A brief lag period followed the initiation of each UF pulse before any resulting transition occurred in the RBV curve. This transition was named, the RBV transition and the time delay, the RBV transition time (T_t). Mean T_t for all UF pulses was 33.4 ± 10.0 seconds. T_t probably represents the transit time of ultrafiltered blood from the dialyser through the bubble trap, venous needle, cardiopulmonary circulation, back through the fistula and arterial needle and finally to the BVM. T_t thus depends on factors such as cardiac output, Q_b , and tubing and vessel dimensions. All estimates of measured and derived RBV parameters were initiated from the point of RBV transition.

Measured Parameters Parameters analysed in a typical relative blood volume profile (see Chapter 4, Experiment 1)

Derived Parameters.

i. Decay constants. (see Chapter 4, Experiment 1)

ii. Linear divergence A sample of relative blood volume data obtained over 1 minute during the UF phase beginning at 30 seconds after the RBV transition was used to compute the relative blood volume linear slope. Fig 1 depicts simulated exponential decay curves with identical coefficients but variable decay constants (Line A, B, C). The area between the linear and experimental curve data for each curve was derived by integration to obtain the net deviation of the experimental UF curve from its extrapolated initial linear slope. This is illustrated schematically in Fig 2a and 2b. This quantity was termed, the **Linear divergence** (%. seconds). This quantity is defined as the net deviation of the RBV decay curve during UF from the

linear decay curve extrapolated from an initial 1 minute RBV slope. The change in Linear divergence as target weight is approached during successive UF pulses in a single patient study is shown in Figure 3.

Statistical Analysis

The statistical tests used were the Students' t-test (paired) for differences between means, the Pearson correlation coefficient to explore the relationships between pairs of variables, and multiple regression analysis to explore the relationships between multiple variables. Chi-squared analysis was used to determine the significance of proportional differences. All analyses were performed using the Systat 9 and Sigma plot statistical software. [see methods]

Results

Mean interdialytic weight gain was 2.19 +/- 0.89 kg. The mean isovolemic UFVs was 470 +/- 142 ml. Mean interdialytic weight gain was 2.19 +/- 0.89 kg.

Correlations with proximity to dry-weight The correlations between the measured and derived relative blood volume parameters during the UF and refill phases and proximity to dry weight (PDW) are shown in table 1. The best correlation was provided by Linear divergence ($r = 0.817$, $p < 0.001$) Figure 4. There were also significant correlations with $\Delta RBV_{UF}/UFV_s$, RBV at initiation of UF pulse, UFV_s , and UF decay amplitude. There were no significant correlations with any of the parameters relating to the refill phase. When these significant factors were examined in stepwise multiple regression analysis, the strongest predictor of proximity to dry weight was Linear divergence ($\beta = 0.527$; $p < 0.001$). Other significant predictors were RBV at initiation of UF pulse ($\beta = 0.242$; $p < 0.001$), UFV_s ($\beta = 0.161$; $p = 0.035$) and τ_{UF} ($\beta = 0.147$; $p = 0.037$). The overall adjusted R^2 was 0.756. There were significant differences between all these parameters together with $\Delta RBV_{UF}/UFV_s$ and UF decay amplitude during UF pulses within 1kg of dry weight and those further from dry weight (table 2). None of the parameters relating to the refill phase were different between these two hydration states.

Determinants of hypotension. There were significant differences between mean values of τ_{UF} , Linear divergence, RBV at UF pulse initiation, refill phase amplitude, and UF decay amplitude, between hypotensive and normotensive UF pulses (Table 3). τ_{UF} and Linear divergence showed the clearest demarcation between hypotensive and normotensive groups (Figure 5), such that all patients with $\tau_{UF} > 22$ and 93.3% of patients with Linear divergence < 85 developed hypotension later during the course of the same UF pulse.

Changes in UF parameters during successive ultrafiltration pulses (Table 4). Linear divergence fell significantly during successive UF pulses of equal volume ($p < 0.001$ for both pulse 2 vs 3 and 3 vs 4). Figure 6 Mean UF decay constant (τ_{UF}) increased significantly between pulse 3 and pulse 4 ($p < 0.001$) but there was no difference between pulse 2 and pulse 3 with respect to this parameter. The 40 % UF pulse (pulse 1) was not used for comparison being of greater volume than the other pulses. Fig 7a and 7b demonstrates some individual case studies of diminishing linear divergence in two patients.

Influence of antihypertensive agents

There was an effect of antihypertensive medication on blood volume decay parameters. ΔRBV_{UF} (7.5 ± 1.5 vs 6.6 ± 1.7 ; $p < 0.013$) and Linear divergence (596 ± 440 vs 350 ± 403 ; $p < 0.011$) were greater in patients taking antihypertensive agents than in those not taking these agents. There were no differences between these groups with respect to any other parameter. However mean PDW and mean UFV_s were also greater in those taking these agents (1088 ± 453 vs 867 ± 452 ml; $p < 0.031$ and 492 ± 93 vs 410 ± 140 ml; $p < 0.002$, respectively). Linear divergence but not ΔRBV_{UF} correlated directly with proximity to dry weight ($r = 0.817$; $p < 0.001$) and both ΔRBV_{UF} ($r = 0.553$; $p < 0.001$) and Linear divergence ($r = 0.462$; $p < 0.001$) correlated directly with UFV_s .

Critical blood volume and hypotension Hypotension was significantly more common in UF pulses commencing with RBV $< 90\%$ of its baseline value ($p = 0.001$). Hypotensive episodes occurred in 64% of UF pulses commencing at this critically low blood volume. However hypotension also complicated 24% of UF

episodes with an initial RBV greater than this level and indeed most episodes of hypotension (16 of the 30 episodes) occurred in patients with an initial RBV >90%. In 479 simultaneous measurements of blood pressure and RBV (Figure 8a, 8b), there was no significant difference between the incidence of hypotension (systolic blood pressure ≤ 90 mm Hg) when RBV was reduced to <90% of the starting value (13 of 176 episodes [7.4%]), and when RBV was maintained at $\geq 90\%$ (16 of 303 episodes [5.3%])

Predictors of Linear divergence

Linear divergence correlated with proximity to dry weight ($r=0.817$: $p<0.001$), and with the measured relative blood volume parameters, RBV ($r=0.312$: $p=0.069$), UFV ($r=0.462$: $p<0.001$), and $\Delta RBV_{UF}/UFV_s$ ($r= -0.452$: $p<0.001$). There was no correlation with ΔRBV_{UF} and IRR. As noted above (Table 3 and Figure 5), Linear divergence was significantly smaller in hypotensive pulses than in non-hypotensive pulses (155 \pm 285 vs 662 \pm 405: $p<0.001$). In multiple regression analysis using these significant factors, the only independent determinant of Linear divergence, was proximity to dry weight ($R^2=0.667$: $p<0.001$).

Predictors of τ_{UF}

τ_{UF} correlated with proximity to dry weight ($r= -0.508$: $p<0.001$) but not with the measured relative blood volume parameters, RBV, ΔRBV_{UF} , UFV, $\Delta RBV_{UF}/UFV_s$, and IRR. As noted above τ_{UF} was significantly greater in hypotensive pulses than in non-hypotensive pulses (21.6 \pm 8.5 vs 12.8 \pm 2.8: $p<0.001$) (Table 3 and Figure 5). The occurrence of a hypotension during a UF pulse was the only significant independent predictor of τ_{UF} in stepwise multiple regression analysis ($R^2 = 0.400$: $p<0.001$).

Discussion

Fluid removal during haemodialysis is a complex process. The response to fluid removal varies during the dialysis session, between dialysis sessions, and between patients. Multiple factors are involved including the prevailing state of hydration, the mode of dialysis, variations in patient physiology and pathophysiology, water purity, dialysis fluid composition and temperature, and medication.

Despite the development of objective tools for assessment of fluid compartments (e.g. caval diameter, bioimpedance), their intradialytic application is limited by numerous factors including measurement artefacts introduced by intradialytic effects such as temperature variations and ionic shifts, difficulties in bedside usage, and their provision of static ‘snapshot’ data on individual fluid compartments [219]. Online Blood volume monitoring on the other hand is easy to use and provides continuous, high-resolution data that can be used to characterise the response to ultrafiltration.

In complex systems such as the pathophysiological response to ultrafiltration, modelling is difficult as multiple factors are involved such as hydration state, sympathovagal balance [104], and momentary local electrolyte and mineral balance. The system may be regarded as a complex arrangement of dynamic elements linked by an integrated array of regulatory mechanisms. When ultrafiltration is initiated, a number of physiological compensatory mechanisms are invoked for blood volume and blood pressure maintenance. Under these circumstances, perturbation analysis may be useful. Using this technique intermittent, short bursts of UF was applied at a high rate with intervening rest periods to probe relative blood volume responses at different states of hydration. UF rates in excess of interstitial refill capacity (1.5 – 2l/hr) were used to maximise the information content of the system response. Stiller and Mann [220] have demonstrated the principle of using intermittent UF to extract information about refilling. This is a potentially powerful experimental tool for haemodynamic analysis in the haemodialysis context, capable of providing information on refill capacity and hydration status of fluid compartments.

Classical models of the circulation make use of two compartments, vascular and interstitial, separated by the capillary membrane interface [221, 212]. These models also implement the physical principles of the Starling force balance mechanism. Blood volume during UF tends to fall exponentially [221]. This exponential decay is a function of a constant UF rate, and a variable rate of tissue refill. In most cases the vascular space must be depleted sufficiently to promote vascular refilling. Once refilling is underway, the blood volume decay tends to “plateau”. Under these conditions the vascular refilling rate approximates the UF rate. If the UF rate is

increased, the plateau occurs at a lower blood volume. If the plateau is still present at the end of UF, this indicates that vascular refilling is still taking place and that dry weight has not yet been achieved.

The study demonstrated that with dryness blood volume declines in a linear fashion as UF proceeds. At a fixed UF rate the depletion in blood volume is likely to occur in a linear fashion when the compartment behaves as a single pool. Approaching linearity I believe indicates the switch from a 2-pool (interstitial and vascular) to an effective single pool blood volume compartment. This is primarily due to absence of refill from the interstitial space but can also result from microcirculatory changes. The latter is probably a compensatory mechanism to excessive UF preceding haemodynamic instability. In essence, the 2-pool system has been reduced to a single vascular pool: the interstitial compartment is effectively 'empty' of readily mobilisable fluid (Figure 9a, 9b).

Antihypertensive therapy was not stopped for the purpose of the study since it was considered important that these studies were performed under conditions pertaining in routine clinical practice. An effect of antihypertensive therapy on Linear divergence, which was greater in patients taking antihypertensive agents than in others, therefore cannot be excluded. The numbers were too small to analyse the effect of one particular group of anti-hypertensives compared to another. However as described above, Linear divergence also correlated directly with proximity to dry weight and UF volume, so perhaps the more likely interpretation for the observed differences in Linear divergence is that patients taking antihypertensive medication were likely to be more volume overloaded than their counterparts not taking such medication.

Whether or not the system is behaving in this linear fashion can be resolved by appeal to simple parameters, which can be derived from serial measurements of relative blood volume during haemodialysis. The UF phase provided the most useful parameters. Refill parameters were much less predictable, presumably because of wide inter-patient variability. Under such conditions simple exponential functions may be of limited use in defining the post UF refill phase parameters. The most promising

parameter was one we termed the Linear divergence. Diminishing Linear divergence during UF was predictive of proximity to achievable dry weight and of the risk of impending hypotension. The only other parameter to approach this in terms of predictive utility was the ultrafiltration decay constant (τ_{UF}), which is also a measure of the deviation from linearity of the UF curve. No directly measured RBV parameter independently predicted proximity to dry weight. The incidence of hypotension was similar whether RBV was less than 90% or higher. Change in RBV during a UF pulse (ΔRBV_{UF}) was no different in hypotensive and in normotensive UF pulses, and no different in UF pulses commencing within 1 kg of dry weight and in those commencing further from dry weight. These findings undermine the case for the routine clinical use of these directly measured parameters and the usefulness of the ‘critical volume concept [216]. Tonelli et al [222] in a study on acute renal failure patients on intermittent HD demonstrated no evidence of patient-specific or universal RBV thresholds that were associated with hypotension. Analysis using the kappa statistic showed that concordance of RBV and hypotension (that is, RBV falling prior to hypotensive episodes rather than rising or remaining stable) was no greater than chance. They emphasize that system behaviour patterns, defined by blood volume decay characteristics may be more powerful predictors of approaching dryness or impending vascular instability rather than static RBV measurement at any given point of time.

Calculation of variability of the blood volume curves around a linear slope as described by Biege et al requires the derivation of a linear regression line from the relative blood volume data of the entire dialysis session [223]. Calculation of the ultrafiltration decay constant (τ_{UF}) described here requires the output of RBV monitoring during a single UF pulse rather than from a whole dialysis session. Our Linear divergence approach has the advantage of using the initial linear slope of the local RBV data to test linearity. This approach is therefore potentially more useful than simple curve characterization. System behaviour patterns at the beginning of dialysis may not represent the system characteristics at later stages of dialysis or ultrafiltration. The information obtained during the initial phase therefore becomes historical. Biofeedback UF control based on predefined BV trajectory at the start of dialysis lacks the capacity to adapt to patient behaviour during UF. [224,

225] In addition the assumption of a constant F cell ratio implicit in relative blood volume monitoring may not be rigorously applicable throughout a whole dialysis session. RBV data from the start of dialysis may not be strictly comparable with that obtained in the later stages because of haematocrit redistribution. Our protocol allows testing the haemodynamics and UF tolerance repeatedly at different stages of ultrafiltration. The linearity in our model therefore is fundamentally different that described in other studies.

The use of slope evaluation to derive absolute blood volume changes during HD has proved difficult in previous studies since redistribution effects may influence it during ultrafiltration [226]. Linear divergence estimation is very sensitive to correct evaluation of slopes. It can only be computed using high-resolution sensors. The refill area calculated from an initial 1-minute slope best defines it. Slope determination must be commenced 30 seconds after RBV transition. This 30-second delay after RBV transition adds precision presumably because it avoids any recirculatory effects on the RBV data. Longer delays, certainly beyond 90 seconds, produce inaccuracies, presumably because refill has commenced. There are other potential problems. There is noise in the RBV data, which could be related to refilling forces, variations in vascular compliance, UF delay, inaccurate or variable UF rates and data artefacts (breathing, machine alarms). Low noise signals ratio, high resolution (estimates of RBV every 3 seconds) and accurate UF rates, are prerequisites, which allow the derivation of parameters such as linear divergence and the ultrafiltration decay constant (τ_{UF}) from serial RBV data.

These experiments have demonstrated that as dry weight is approached, blood volume decline during UF switches from exponential to linear decay. This can be described in terms of a number of parameters, which may be derived from serial relative blood volume data. Linear divergence was the most promising. Diminishing Linear divergence during UF was predictive of proximity to achievable dry weight and of the risk of impending hypotension. Improved control algorithms, incorporating such parameters, which can be assessed repeatedly during dialysis, may help optimise UF prescription, improve haemodynamic stability, and facilitate achievement of the best possible post-dialysis target weight.

Table 1. Correlations (Pearson) between the measured and derived relative blood volume parameters during the ultrafiltration (UF) and refill phases and proximity to dry weight

Parameter	Correlation coefficient	p-value
RBV at initiation of UF pulse (%)	0.479	<0.001
ΔRBV_{UF} (%)	0.029	NS
UFV _s (ml)	0.475	<0.001
$\Delta RBV_{UF}/UFV_s$ (%/ml)	-0.506	<0.001
UF decay amplitude (b)	0.401	<0.001
τ_{UF}	-0.508	<0.001
Linear divergence (%. sec)	0.817	<0.001
IRR (%/min)	-0.023	NS
Refill phase amplitude	0.182	NS
τ_{ref}	-0.115	NS

RBV (%) relative blood volume, ΔRBV_{UF} (%) reduction in RBV, UFV_s volume of step ultrafiltration pulse, IRR initial refill rate, τ_{ref} refill constant, τ_{UF} ultrafiltration decay constant

Table 2 Chi squared analysis comparing measured and derived relative blood volume characteristics of ultrafiltration pulses within 1kg (n= 49) and those \geq 1kg (n=41) from dry weight (mean \pm standard deviation) during UF and refill phases

Parameter	UF boli < 1kg from dry-weight	UF boli \geq 1 kg from dry-weight	p-value
RBV at UF pulse initiation (%)	91.4 \pm 4.1	95.3 \pm 3.2	<0.001
ΔRBV_{UF} (%)	7.2 \pm 1.7	6.9 \pm 1.5	NS
UFV _s (ml)	420 \pm 122	500 \pm 109	0.001
$\Delta RBV_{UF}/UFV_s$ (%/ml)	0.018 \pm 0.005	0.014 \pm 0.003	<0.001
UF decay amplitude (b)	72.4 \pm 13.5	81.7 \pm 13.2	0.001
τ_{UF}	18.6 \pm 8.0	12.3 \pm 2.2	<0.001
Linear divergence (%.sec)	202 \pm 277	809 \pm 359	<0.001
IRR (%/min)	0.77 \pm 0.38	0.83 \pm 0.41	NS
Refill phase amplitude	4.2 \pm 2.5	4.1 \pm 1.8	NS
τ_{ref}	0.2 \pm 0.53	0.15 \pm 0.11	NS

RBV (%) relative blood volume, ΔRBV_{UF} (%) reduction in RBV, UFV_s volume of ultrafiltration pulse, IRR initial refill rate, τ_{ref} refill decay constant, τ_{UF} ultrafiltration decay constant

Table 3 Chi-squared analysis comparing UF and refill characteristics between hypotensive (n=30) and normotensive UF pulses (n=60)

Parameter	Hypotensive UF pulses	Normotensive UF pulses	p-value
RBV at UF pulse initiation (%)	90.5 ± 4.2	94.6 ± 3.5	<0.001
$\Delta\text{RBV}_{\text{UF}}$ (%)	7.4 ± 1.8	6.9 ± 1.5	NS
UFV ₁ (ml)	457 ± 123	457 ± 123	NS
$\Delta\text{RBV}_{\text{UF}}/\text{UFV}_1$ (%/ml)	0.017 ± 0.005	0.016 ± 0.004	NS
UF decay amplitude (b)	71.2 ± 12.9	79.4 ± 13.9	0.007
τ_{UF}	21.6 ± 8.5	12.8 ± 2.8	<0.001
Linear divergence (%.sec)	155 ± 285	662 ± 405	<0.001
IRR (%/min)	0.86 ± 0.45	0.76 ± 0.35	NS
Refill phase amplitude	3.4 ± 1.1	4.8 ± 2.4	0.001
τ_{ref}	0.25 ± 0.69	0.14 ± 0.10	NS

(mean ± standard deviation) For key: see Table 2

Table 4 Comparison of mean Linear divergence and mean UF decay constant (τ_{UF}) during successive UF pulses of equal volume in the 30 studied patients.

Parameter	UF pulse 2	UF pulse 3	UF pulse 4
Linear divergence (%.sec)	764.5 (454.1)	546.6 (312)*	155.5 (285.4)*
τ_{UF} (UF decay constant)	12.05 (2.67)	13.48 (2.87)	21.64(8.53)*
UF volume (ml)	456.1(124.1)	457.3(123.6)	456.5 (123.2)

* denotes significant difference (p < 0.001) from same parameter in preceding UF pulse.

(B) Determination of the effect of variable ultrafiltration rate on blood volume profile characteristics

The Ultrafiltrate volume as described in Chapter 4 has a powerful effect on the relative blood volume changes. This may be as a consequence of the variable quantity of fluid removed, patient specific factors such as variations in plasma refill rate or differences in the rate at which fluid is removed (UFR or ultrafiltration rate). This third phase experiment was designed to investigate the latter mechanism independent of the other potential influences.

The aim of the study was to measure the effect of different UF rates alone on relative blood volume characteristics in the same patient using an identical UF profile during otherwise identical two consecutive dialysis sessions.

EXPERIMENTAL SET UP 3

Variable volume, varying rate step ultrafiltration (2 v 3 l/hr)

Paired studies were performed in 5 patients with stable interdialytic weight gains (IDWG) on consecutive dialyses using an identical dialysis prescription and UF profile (Experiment 1) but at two differential UFR of 2 and 3 litres /hr (Fig 10)

Results

Varying volume, varying rate step ultrafiltration (10 treatments)

Higher UF rates (3 litres/hr) were associated with higher ΔRBV_{UF} [6.3 +/- 2.0 % v 5.2 +/- 1.9%] ($p < 0.001$) and lower linear divergence [422 +/- 565 %. sec v 641 +/- 875 %. sec] ($p < 0.05$) when compared with UF rates of 2 litres /hr in the same patient at similar states of hydration under identical UF profile and blood flow rates. (Table 5)

Discussion

The experiment demonstrates that in standard high flux dialysis, the UF rate i.e. ultrafiltration time duration, has a significant effect on the blood volume decay parameters independent of the volume of the ultrafiltrate or other influences in the same individual under otherwise similar conditions. At high UF rates, higher relative blood volume decay and reduced linear divergence (both predictors of hypotension) may predispose the patient to haemodynamic instability.

The effect of high UF rates on haemodynamic parameters depend on its interaction with refill capacity and hydration states. Longer treatment times or frequent therapy can lower total ultrafiltration rate required to maintain patient well being and preserve vascular stability. [227, 228] On the other hand, in conventional dialysis, varying UF rates to match the changing refill capacity during dialysis might allow a more physiological ultrafiltration schedule and reduce dysequilibrium. This however requires i) an understanding of how refill rates tend to vary in relation to blood volume and hydration states ii) an ability to quantify refill objectively and subsequent use of systems with control feedback device. These aspects have been examined at a later stage in Chapter 8.

Table 5 Paired t test comparing means of the RBV parameters at two differential UF rates (3 and 2 l/hr) but otherwise identical dialysis session in 5 subjects Mean (+/- sd)

UF parameters	2l/ hr	3 l / hr	P value
UF_vol (ml)	331 (135)	336 (143)	ns
d_RBV (%)	5.18 (1.86)	6.3 (1.99)	< 0.001
tau UF	13.9 (9.9)	8.31 (6)	< 0.05
Linear divergence	641 (875)	422 (565)	< 0.05

Figure 1

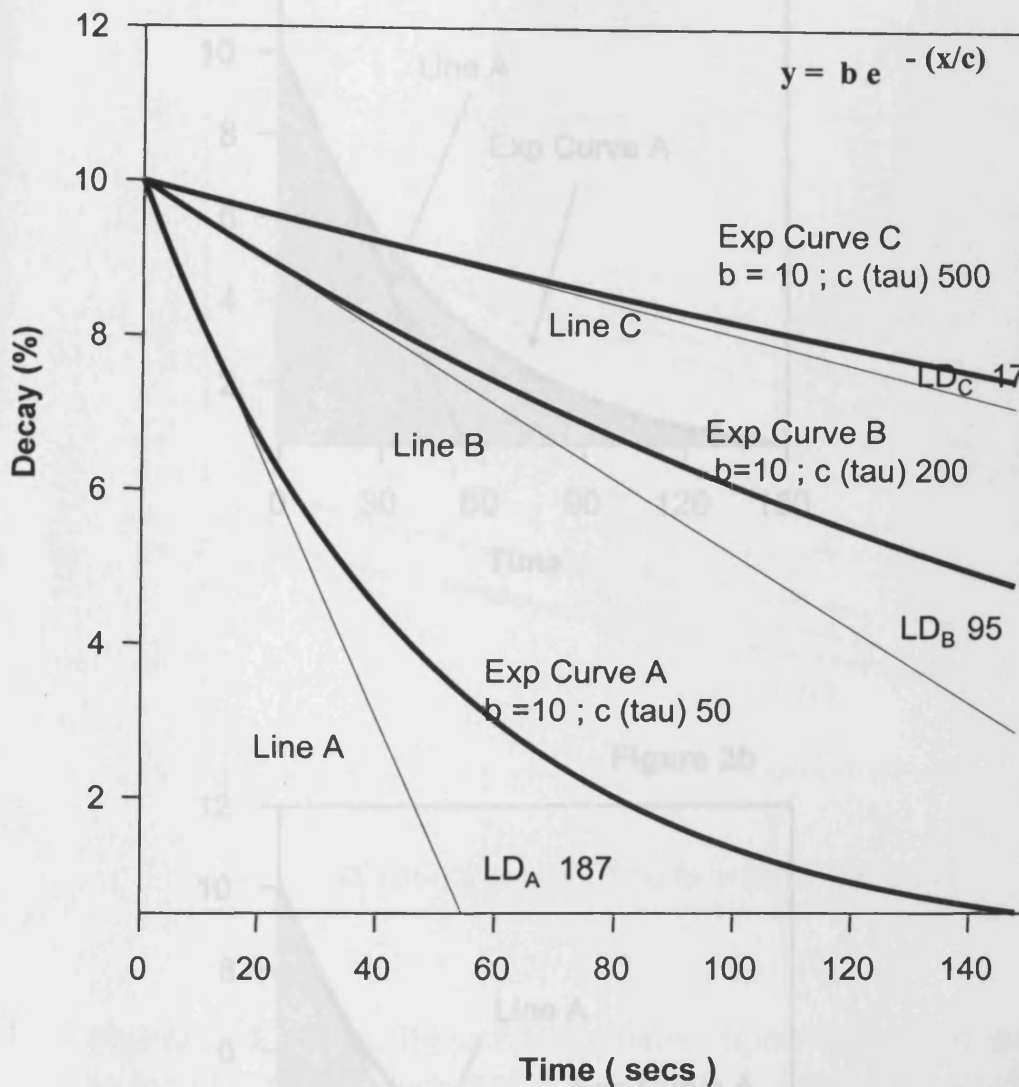


Figure 1 Simulation of the single exponential [$y = b e^{-(x/c)}$] decays (Exp curves A, B, C) with identical coefficient [b (amplitude) = 10] and varying decay constant (c) [c (rate constant) = t (tau)]. The equivalent parameters on a UF induced exponential decay curves are $c = t_{UF}$, and $b = \text{UF decay amplitude}$. Lines A, B and C represent predicted linear decay for each curve. LD (Linear divergence %. sec) represents the integrated area between the exponential and linear decay for each curve. Exp curve C is defined by a higher c (tau) and diminishing Linear divergence.

Figure 2a

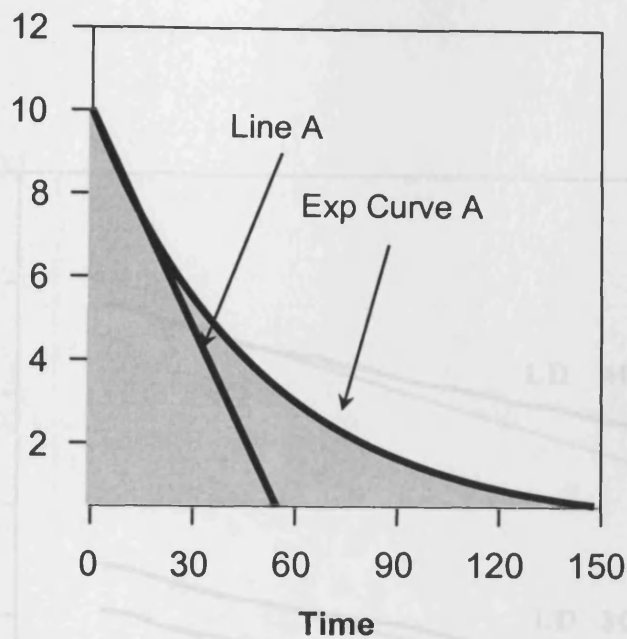


Figure 2b

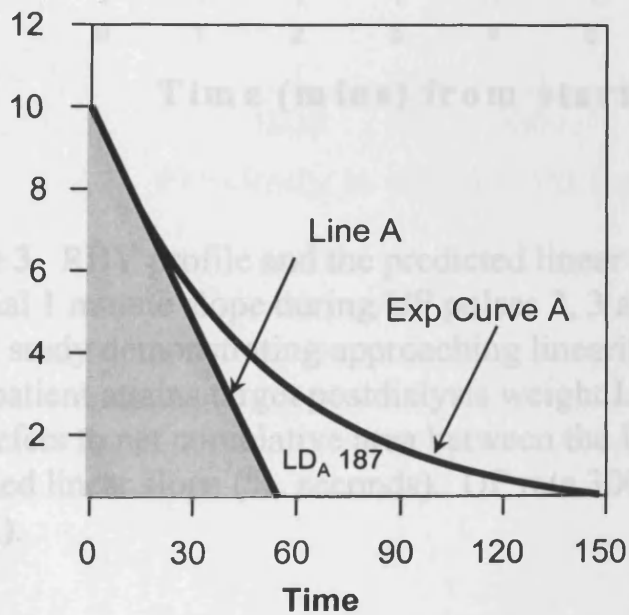


Fig 2a, 2b Demonstration of the derivation of LD (Linear divergence) for Exp curve A. Linear divergence (LD) is the difference between the integrated areas under the Exp curve A (shaded area Fig 2a) and Line A (shaded area Fig 2b).

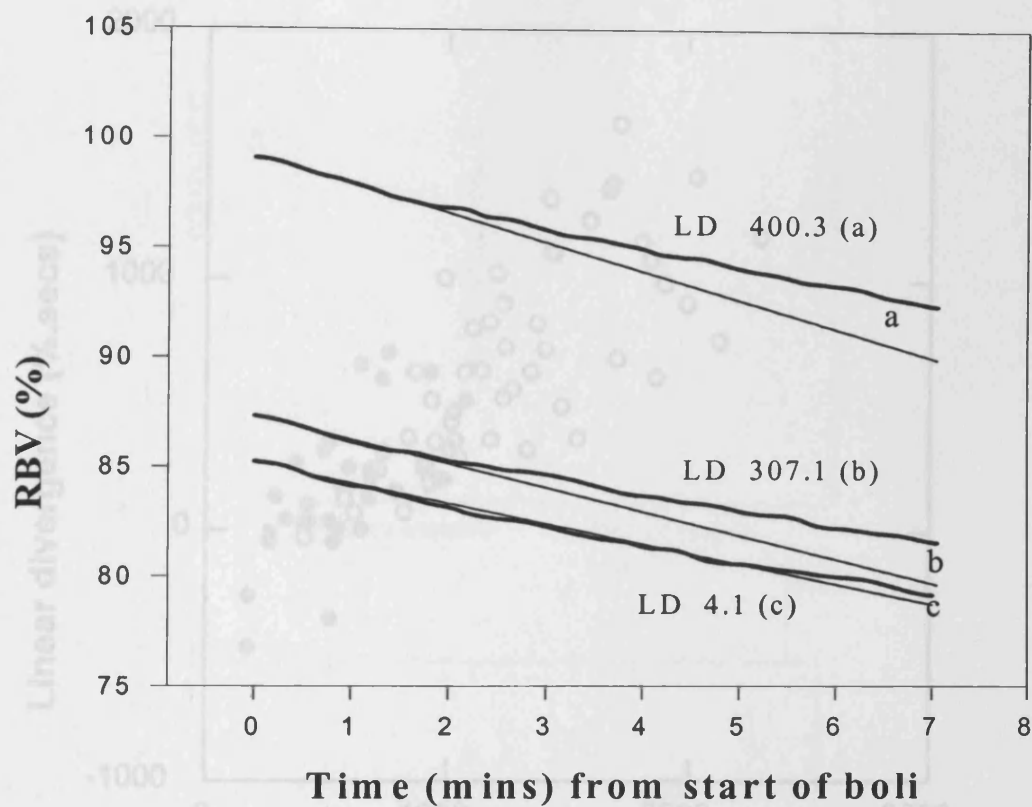


Figure 3 RBV profile and the predicted linear decay derived from an initial 1 minute slope during UF pulses 2, 3 and 4 in a single patient study demonstrating approaching linearity (final pulse c) when patient attains target postdialysis weight. Linear divergence (LD) refers to net cumulative area between the UF profile and the predicted linear slope (%. seconds). UF rate 3000ml/hr, UF volume 350 ml).

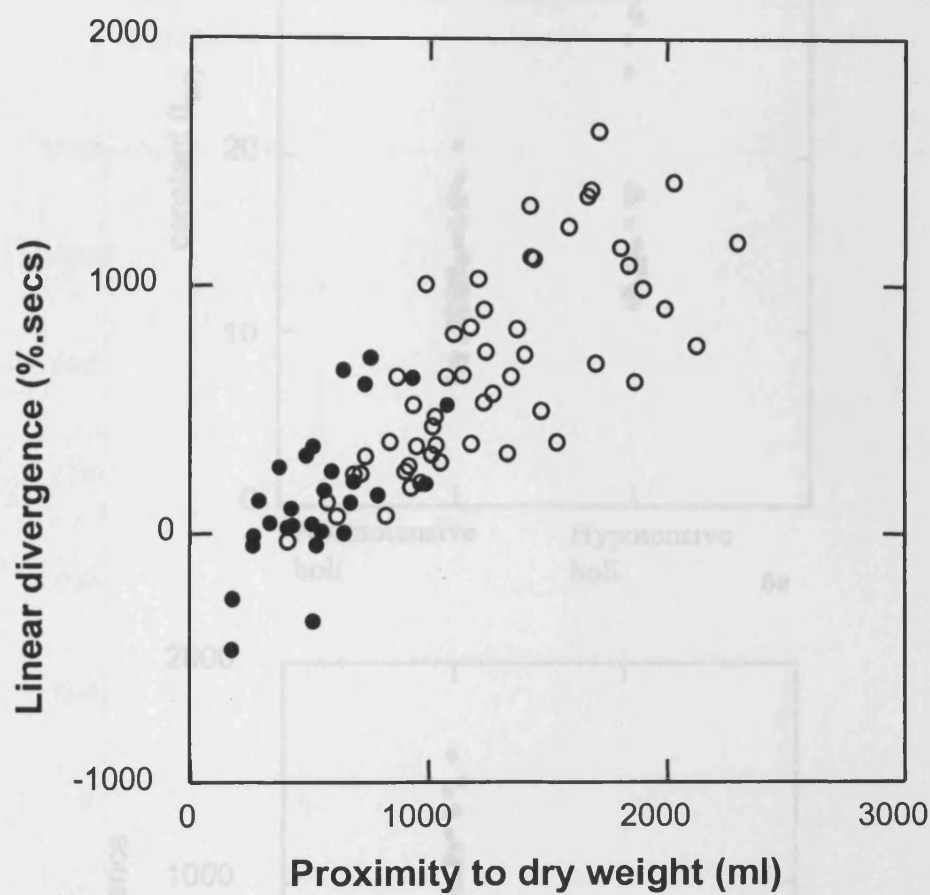


Figure 4 The scatter plot of linear divergence at isovolemic UF pulses with distance from dry weight in thirty subjects. ($r = 0.817$; $p < 0.001$) Bold circles indicate hypotensive UF pulses (systolic < 90 mmHg).

Figure 5 Comparison of ultrafiltration decay constant (t_{UF}) [5a] and Linear divergence [5b] between hypotensive and normotensive pulses ($p < 0.001$)

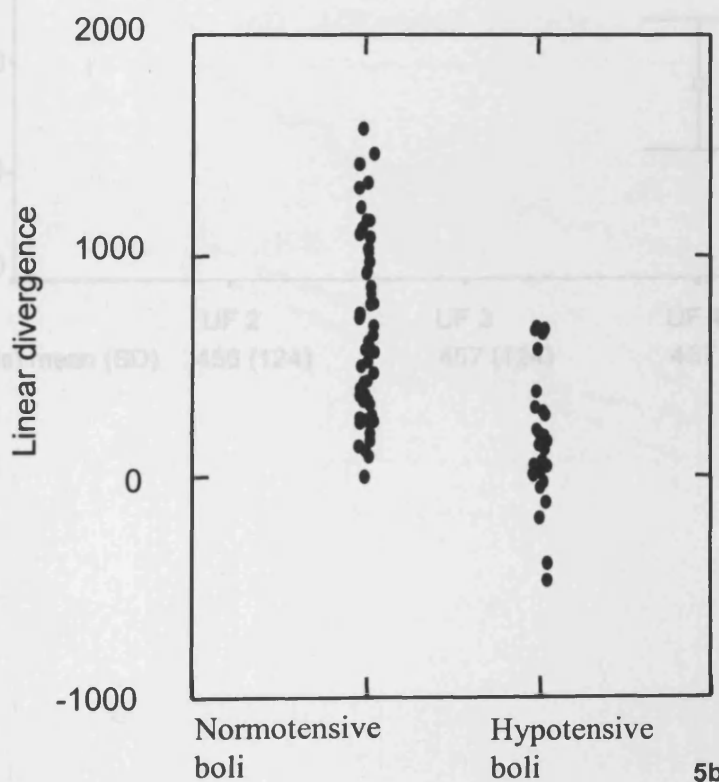
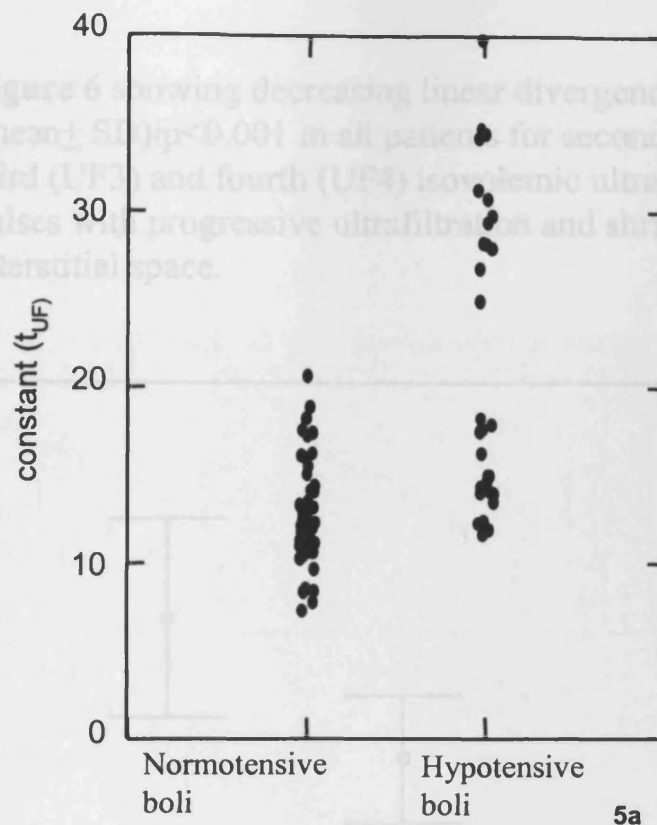


Figure 5 Comparison of ultrafiltration decay constant (t_{UF}) [5a] and Linear divergence [5b] between hypotensive and normotensive pulses ($p < 0.001$)

Figure 6 showing decreasing linear divergence (mean \pm SD) $p<0.001$ in all patients for second (UF2), third (UF3) and fourth (UF4) isovolemic ultrafiltration pulses with progressive ultrafiltration and shrinkage of interstitial space.

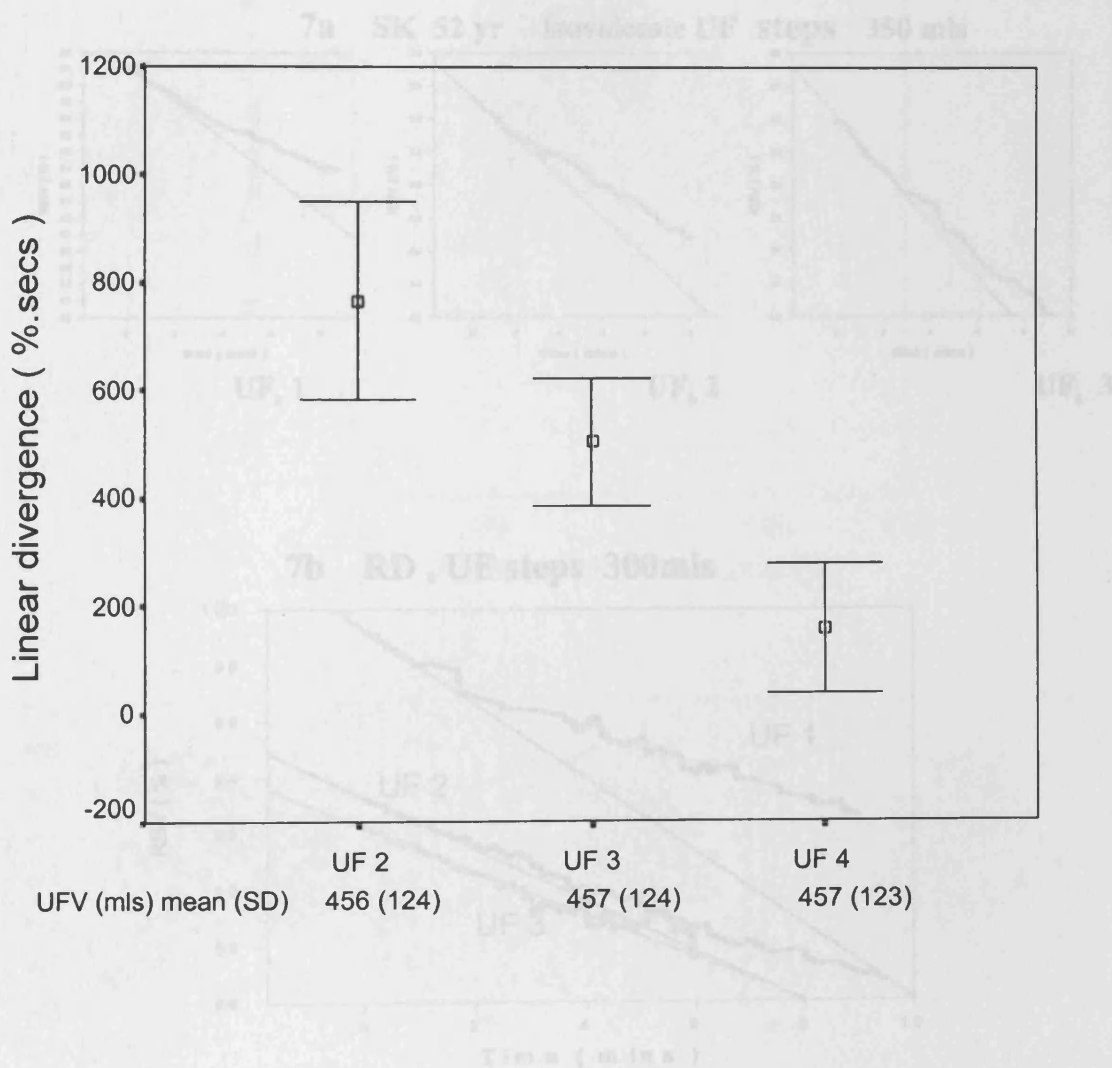
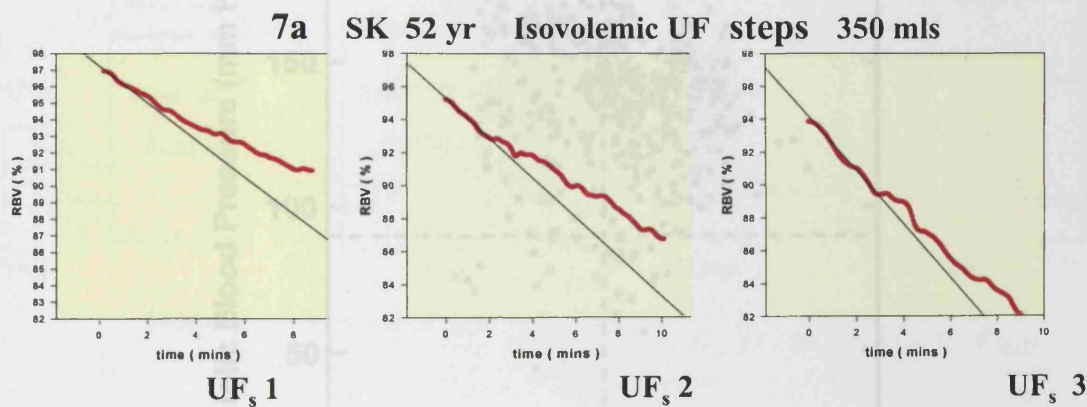


Figure 7a, 7b Patient SK and RD on dialysis with intermittent UF demonstrating Linear divergence at differing states of hydration using identical UF pulse



7b RD , UF steps 300mls

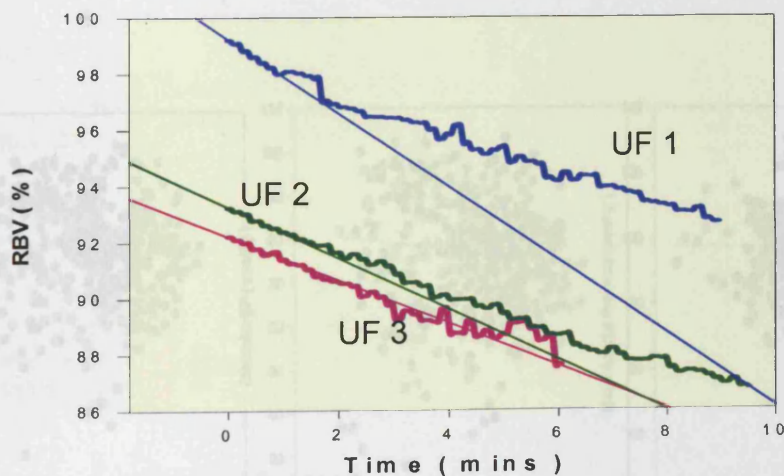


Figure 8b. Correlation of blood pressure (systolic, diastolic and mean BP) with Relative blood volume (%) and Haematocrit (%)
 $p=0.035$ ($r = 0.12$) $n = 458$

Figure 8a Scatterplot of simultaneous measurements of systolic, diastolic and mean blood pressure (mmHg) and relative blood volume (%) [n=479] showing no significant difference in the incidence of hypotension (systolic BP<90mmHg) between RBV \leq 90% and RBV > 90%.

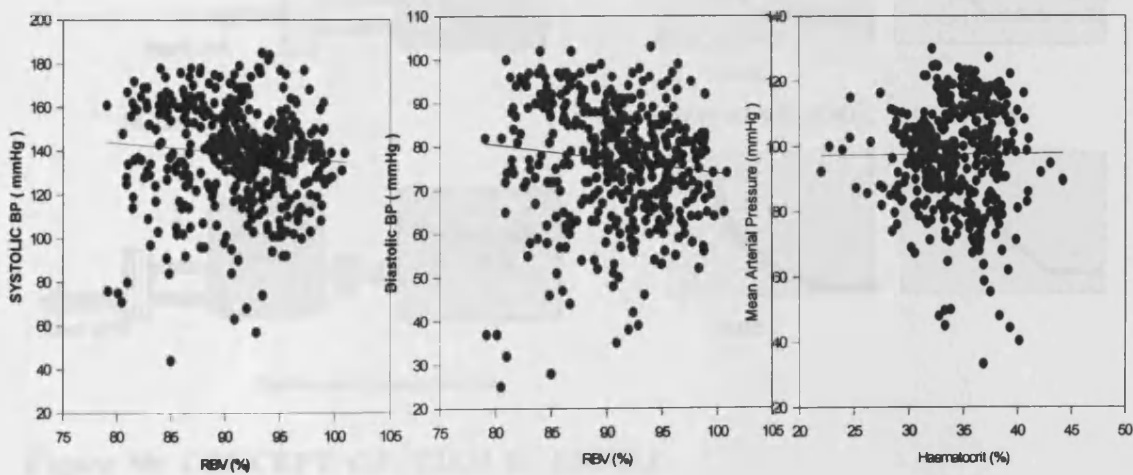
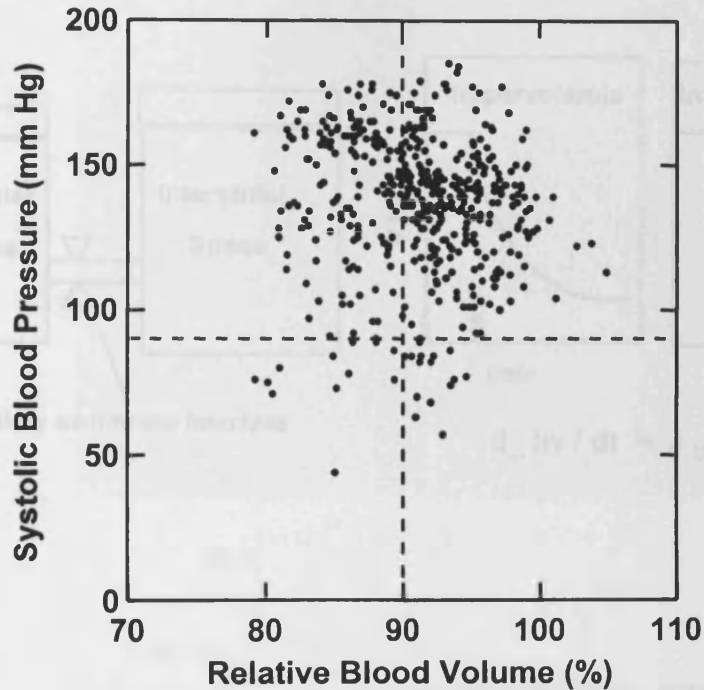


Figure 8b. Correlation of blood pressure (systolic,diastolic and mean BP) with Relative blood volume (%) and Haematocrit (%)
 $p=0.035$ ($r = 0.12$) $n = 458$

Figure 9a A hypothetical model of an open-ended two pool extracellular compartment [A] displaying single pool characteristics and linear decay and [B] on approaching dryness in the presence of inadequate refill during ultrafiltration
 d_{bv} / dt change in blood volume J_{UF} = ultrafiltration force J_{REF} = plasma refill

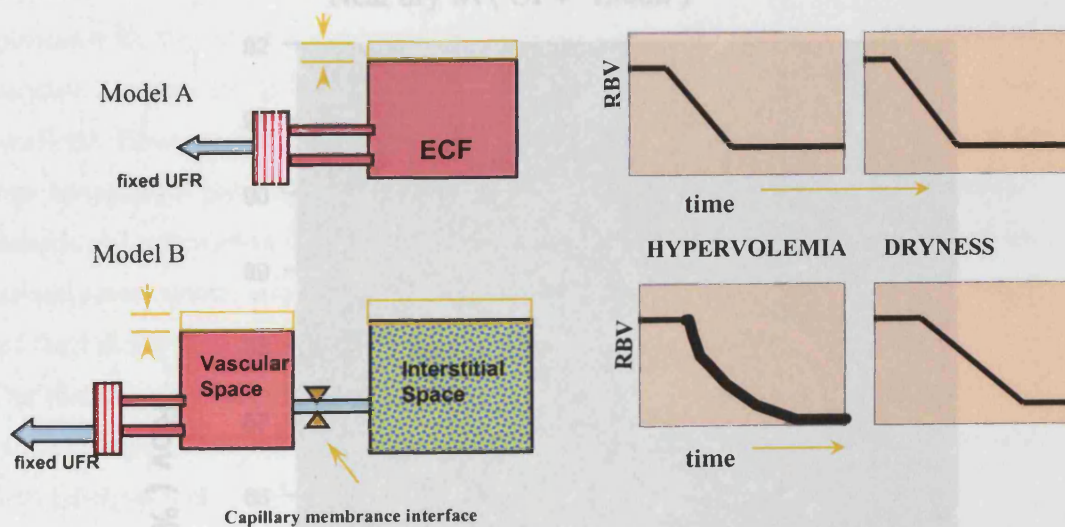
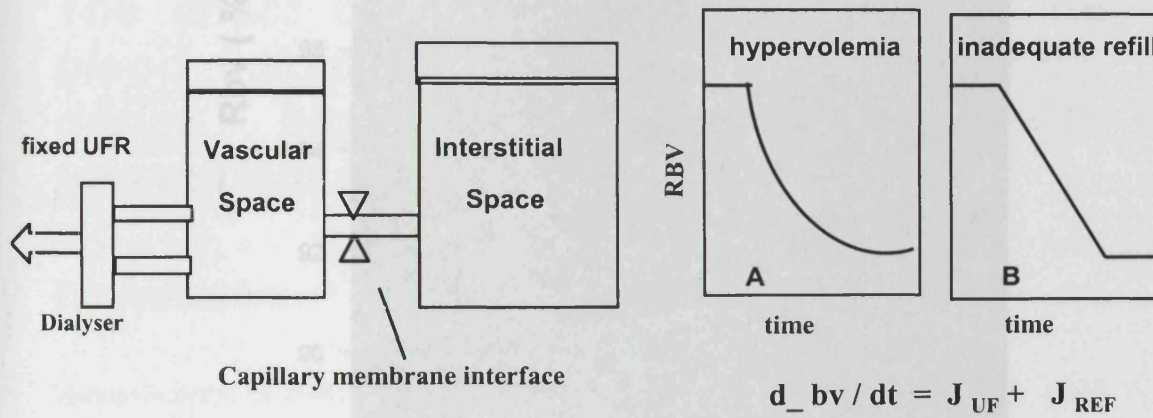


Figure 9b CONCEPT OF TISSUE REFILL

Model A single pool model (open ended)

Model B 2- pool model (open ended) The inserts on the right hand side conceptualize the predicted blood volume decay during UF based for the respective model on the left hand panel during hypervolemia and near interstitial dryness

Figure 10 Subject PB undergoing identical UF pulses at two differing states of hydration on two consecutive dialysis sessions at 2l/hr and 3l/hr showing the differences in the RBV profile

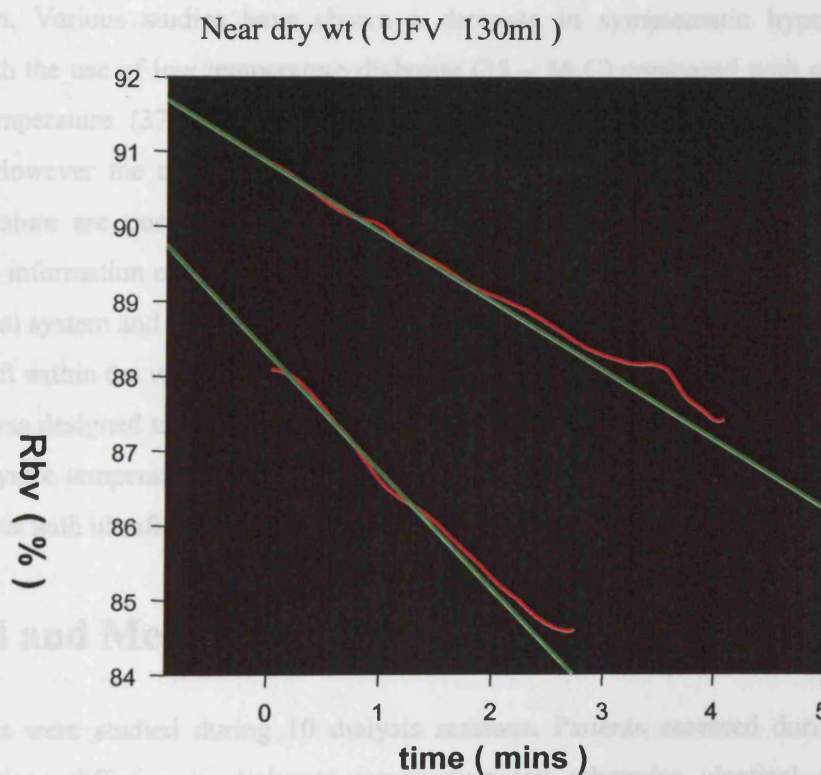
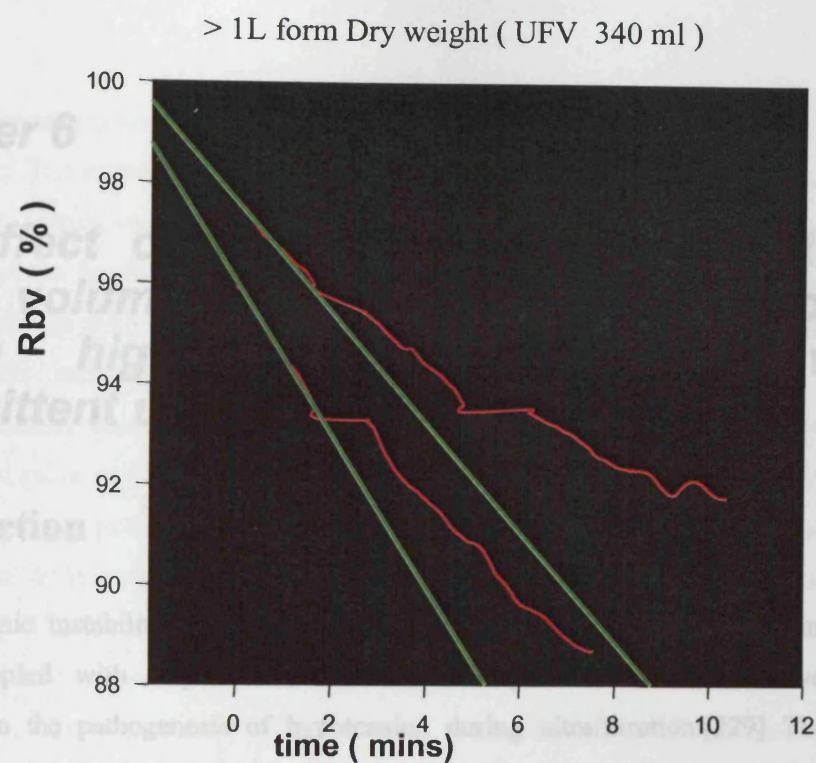


Figure 10 Subject PB undergoing identical UF pulses at two differing states of hydration on two consecutive dialysis sessions at 2l/hr and 3l/hr showing the differences in the RBV profile

Chapter 6

The effect of differential heat gradient on blood volume and haemodynamic stability during high efficiency dialysis with intermittent ultrafiltration

Introduction

Haemodynamic instability frequently occurs during haemodialysis. Decline in blood volume coupled with impaired cardiovascular response mechanisms have been implicated in the pathogenesis of hypotension during ultrafiltration.[229] The latter phenomenon can be influenced by temperature flux incurred during dialysis and ultrafiltration. Various studies have shown a decrease in symptomatic hypotensive episodes with the use of low temperature dialysate (35 – 36 C) compared with standard dialysate temperature (37 – 37.5 C).[230] Cooler dialysis has been found to be beneficial. However the counterregulatory mechanisms that operate with alterations in core temperature are poorly understood.[231] Studies examining the haemodynamic changes and information on energy balance, energy transfer between the patient and the extracorporeal system and the effect of thermal energy transfer on blood volume changes and fluid shift within the vascular compartment are rare. [232]

This study was designed to observe the cardiovascular and thermoregulatory response to varying dialysate temperature, on blood volume and haemodynamic variables and skin during dialysis with ultrafiltration.

Protocol and Methods

Five subjects were studied during 10 dialysis sessions. Patients assessed during two dialysis sessions differing in dialysate temperature but otherwise identical dialysis prescription with respect to time, blood flow, clearance and ultrafiltration. The treatment

sessions for warm (mean 37.4 C) and cool dialysis (mean 35.3 C) was performed in random order. The ambient temperature varied between 22 - 23 C. Each patient served as their control. During each session the temperature was monitored at the arterial and venous side of the fistula as well as the energy transfer in the extracorporeal circuit every 10 seconds. Blood pressure and heart rate was monitored every 10 min by an automatic oscillometric BP monitor built in to the dialysis machine.

Dialysis treatment was performed at the same time and day of the week to keep ultrafiltration gains as close to identical as possible. All patients were ultrafiltered to clinical dry weight. Intermittent UF was applied after an isovolemic period using an initial bolus of 40 % weight gain and then subsequent equal boli of 20% with intervening rest periods. (Protocol B Methods). Most patients developed relative or absolute hypotension at the end of UF. Patients were not allowed to eat or drink during dialysis. Renal disease for the five patients studied were caused by Diabetes, chronic pyelonephritis, hypertensive nephrosclerosis, obstructive uropathy and neuropathic bladder. All subjects had near identical weight gains. No changes were made to their medication Table 1 All patients gave informed consent . Ethical approval obtained prior to the study.

Body temperature was assessed online during HD by the Fresenius BTM module, [Methods] which measures temperature at the arterial side of the fistulae and calculates the central venous blood temperature by correcting for fistula and Cardiopulmonary recirculation. This is also referred to as the Core temperature. The correction is necessary because the arterial blood temperature is determined by the core temperature and the temperature of the recirculated venous blood. Recirculation is measured by the BTM with a temperature bolus produced by a change in blood temperature. Predialytic core temperature BTM is the first reliable temperature obtained by the BTM after the start of dialysis usually within the first 5 mins. The accuracy of the core temp BTM is less than 0.1 C as given by the manufacturer (BTM Fresenius Medical Care Bad Hamburg Germany) Correlation between core temperature BTM and tympanic temp is 0.75 ($p < 0.05$) [233] Warming of a patient's blood can occur both through vasoconstriction and an inappropriately high dialysate temperature. Surface **skin temperature** was measured by

a thermal probe applied to the skin surface over the forehead.

Measurement of **Relative blood volume** RBV was performed using Fresenius ultrasonic BVM monitor. Plasma within blood is the most electrically conductive substance in the body. The changes in electrical conductivity of the thorax, due to pulsate flow of blood through the segment provides the basis for thoracic bioimpedance technology. **Thoracic bioimpedance (BoMed)** Cardiodynamic monitor (NCCOM3-R7) was used to determine haemodynamic parameters of cardiac output, stroke index, end-diastolic volume during a cardiac cycle. [see Methods]

Definitions

Energy transfer is defined as the amount of thermal energy, which was transferred from the ECC to the patient or vice versa. A positive value indicates a net energy transfer from the ECC to the patient and a negative value indicates transfer from the patient to the ECC. Energy transfer in kilojoules is also expressed in watts (1 watt = 3.6 kj/hr). T_{art} and T_{ven} was assessed by continuous temperature monitoring at the arterial and venous side of the ECC by an air filled head with a platinum sensor (BTM), Blood Temperature Monitoring ; Fresenius) around the arterial and venous catheters.

Energy transfer was assessed by continuous measurement of temperature in the arterial and venous side of the extracorporeal system according to the formula :

$$E = C \times \rho \times Q_b \times t \times (T_{ven} - T_{art})$$

C = specific thermal capacity (3.64 kJ/kgC)

ρ = density of blood(1052 kg/m³)

t = time in hr

Q_b = blood flow rate

Resting Energy expenditure (REE) was predicted by Harris and Benedict Eqn

Men REE = $66 + 13.8W + 5H - 6.8 \text{ Age}$ (kcal/24hr)

Women REE = $655 + 9.7W + 1.8H - 4.7 \text{ Age}$ (kcal/24hr)

SVR was derived from cardiac output and Mean arterial blood pressure

Results

A total of seven episodes of symptomatic hypotension (blood pressure was <90 mmHg) requiring intervention were observed, five during warm dialysis and two during cool dialysis. A typical hypotensive episode during a session with heat gain depicted in Fig 1. Step ultrafiltration induced rise in core Temperature is demonstrated in Fig 2. No episodes of symptomatic hypotension occurred during the HD session which achieved 35°C venous temperature. UF rate and net weight loss were not different between the paired treatments.

Core temperatures

Core temperature remained stable or fell during treatment with cool dialysate temperature but increased in all subjects during the use of warm dialysate. The differences in the core temperature between the start and end of dialysis during was significantly different between warm and cool dialysate treatment (Table 2) and had a significant negative correlation with predialytic core temperature. ($r = -0.63$, $p < 0.05$) Fig 3. A mean rise of 0.7°C body temperature in the hypotensive sessions was observed compared to 0.4°C in the stable BP group. Fig 4 depicts core temperature profile in three subjects showing stable or relative cooling during cool dialysate but heat accumulation during warm treatment with maximum heat gain in the subject with lower initial core temperature (middle inset).

Haemodynamic variables and energy transfer with intermittent UF

- **Initial Isovolemic dialysis**

Prior to the onset of UF during the initial isovolemic phase lasting approximately 20 – 30 mins the differential heat gradient led to significantly higher rate of cooling with cool dialysis even in the absence of Ultrafiltration (Table 3a) Cardiovascular variables including RBV were similar except for except systolic, mean and diastolic BP which were better preserved with cooler dialysate.

- **During HD with intermittent UF**

Subsequently throughout HD with intermittent UF the haemodynamic observations showed better preservation of systolic, diastolic and mean arterial pressure ($p < 0.001$) with fewer hypotensive episodes during dialysis with cooler temperatures. The total UF volume removed were similar. BP reduction was associated with low Cardiac output, stroke volume and RBV (Table 3b). The decline in BP was not related to the predialytic body temperature ($r = 0.27$ $p = \text{ns}$).

The average cardiac output, stroke index, end diastolic volume, peak ejection flow, RBV and calculated resistances were not significantly different between paired treatments. (Table 4) There was a significant negative relationship between RBV and calculated peripheral resistance ($P < 0.01$, $R = -0.34$). (Fig 5). Fig 6 demonstrates relationship of RBV measurements during a single dialysis session in a subject with other measured cardiac parameters. SVR was significantly correlated to the MAP, volume of ultrafiltrate and time on dialysis, skin core gradient ($r = 0.33$ $p < 0.001$) and weak negative correlation to RBV, core temperature and blood flow rate ($r = -0.37$ $p < 0.01$).

Using multiple regression, the systolic BP was best predicted by Cardiac output, stroke index, end diastolic volume and SVR and thermal variables of energy transfer and core skin differential gradient ($R = 0.8$, $R^2 = 0.64$, $p < 0.001$) The MAP was best predicted by Core temperature in addition to the above variables ($R = 0.81$, $R^2 = 0.66$, $p < 0.001$). Relative blood volume predictors were ultrafiltered volume removed, cardiac output, stroke index, SVR ($R = 0.74$, $R^2 = 0.55$, $p < 0.01$) but not the thermoregulatory factors. SVR was best predicted by the Core temperature and skin core gradient ($P < 0.01$

- **Energy transfer**

During cool dialysis there is significantly higher net heat loss from patient to the ECC. Lower dialysate temperatures ($T_{\text{dia}} 35.3$) are associated with cooler peripheries, lower core temperatures and increased heat loss. The increase in core temperature during warm dialysis occurred despite a relatively small net energy transfer from patient to the extracorporeal circuit. The mechanisms are not clear. Although peripheral resistances seem better preserved in cooler dialysis they do not reach significance in these subjects.

Between UF and non UF phase the temp flux and skin regulation were not significantly different.

By stepwise multiple regression, energy transfer was best predicted by Core temperature T art, blood flow rate, core-skin gradient. ($r = 0.58$, $R^2 = 0.34$, $p < 0.001$). The cooling effect was diminished when high blood flow rates were used with warm dialysate temperatures. (Fig 7) The Linear divergence were no different at identical boli with two different dialysate temperature.

The skin heat regulation seems to play a particularly significant role in energy transfer and thermal balance during warm and cool dialysis. It also bears a significant relationship with haemodynamic variables of Systolic BP , MAP, CO, and SVR but not to RBV.

(Table 5) (Fig 8) This constitutes an important nonextracorporeal route of heat exchange. (Fig 9)

Discussion

Body temperature control during dialysis is governed by thermal energy balance and heat capacity of the human body. The heat gain during dialysis can lead to a heat stroke like phenomenon leading to hypotension. The contribution of the vasoconstriction and heat exchange by the extracorporeal circuit can be estimated as a function of energy transfer between patient, ECC, dialysate temperature and ultrafiltration.

The degree of cooling and hypotension observed in this study are similar other studies where body temperature changed by 0.51°C when cool dialysate was used. [234]

Resting energy expenditure of a 70 kg adult is 116 watts. This is normally dissipated to the environment by evaporation (22%), conduction(18%) and radiation (60%). Thermal regulation tries to keep the body temperature close to an individual set temperature by changing heat loss through vasodilatation or vasoconstriction. If the temperature cannot be kept in a narrow range, hypothalamus centres initiate sweating or shivering. The core temperature at which sweating starts depends on the set temperature and the skin temperature.

The intradialytic thermal balance is influenced by the extracorporeal circuit (ECC). The dialyser is a perfect heat exchanger. Blood leaves the dialyser in thermal equilibrium with the dialysate. The energy balance in the dialyser is expressed as

$$(T_{art} - T_d) (Q_b - UFR) 3.64 \times 1.052$$

Specific heat of whole blood 3.64 J/g density = 1.052 g/ml at 37 C

The energy balance for a temperature difference of 1C at 400ml/min is 25 W. There is an additional loss of energy in the blood venous line. At blood flows > 100 ml/min the loss depends on the blood temperature difference and length of PVC tubing. For 20 C environment temperature and standard PVC blood tubing of 2.8 m with internal dia 4.5 mm the energy loss is approximately 10 W. [234, 235]

The accumulation of heat during dialysis can be prevented by heat loss across the extracorporeal circuit. This can be enhanced using a cooler dialysate temperature. The study shows that at a dialysate temperature of between 37 – 37.5 C ECC heat loss is minimal and can lead to a rise in core temperature leading to haemodynamic instability. This accumulation of heat despite a small net heat loss must be due to the effect of dialysis and UF on body temperature regulation. The mechanisms remain speculative. [236] Hypovolemia as during UF would cause baroreflex cutaneous vasoconstriction which can result in an increase in core temperature if unregulated, antagonising the response to hypovolemia. The body's ability to reduce cutaneous blood flow is a valuable means of increasing peripheral resistance and maintaining blood pressure despite decreasing blood volume. This may impair thermal balance by reducing transcutaneous heat loss. This in addition to a rise in metabolic rate during HD can cause a rise in core temperature. If the latter reaches a critical level, peripheral vasodilatation may occur dissipating heat but also causing instability of blood volume.

At temperatures of 35 – 35.5 there is significant heat loss to counter any heat gain during HD. A specific dialysate temperature is perhaps less important than the blood dialysate temperature gradient and predialytic patient temperature in quantifying the heat exchange. Body temperature changes during dialysis depend on the difference between the predialysis core temperature and the dialysate temperature. Considering all effects an

average intradialytic heat flux can vary between ± 80 W. Skin circulation is controlled by volume and temperature defense mechanisms.

When heat loss is minimal or neutral through extracorporeal routes, rise in core temperature indicates net heat gain during dialysis, which must be generated by metabolic production, dialytic process and ultrafiltration. Haemodialysis and UF procedure therefore affects core temperature regulation. The removal of heat by the extracorporeal circuit and autoregulatory mechanisms attempt to lower core temperature. The mean net energy loss from patient to the extracorporeal circuit in this study is approximately 35 % resting energy expenditure during cool dialysis. This is accordance with some previously published studies. [237] The mean core temperature despite such heat loss remained at times constant. This demonstrates the heat accumulation during dialysis. The cool dialysis prevents an increase in core temperature in all patients.

Skin blood flow (SkBF) in humans is primarily controlled by thermoregulatory reflexes and to some extent the cutaneous circulation is also controlled by reflexes of nonthermoregulatory origin. The extent to which the cutaneous circulation participates in baroreceptor-mediated reflexes and in the reflexes associated with exercise is debatable. [238] Exercise for example elicits both thermoregulatory and nonthermoregulatory reflexes. The overall conclusion is that thermoregulatory control of SkBF is subject to modification by or competition from several other sources. The fundamental pattern for control of SkBF is described by the threshold and slope of the SkBF-internal temperature relationship. Reflex effects of skin temperature act to shift the threshold of this relationship such that lower levels of skin temperature are associated with higher threshold internal temperatures at which cutaneous vasodilation begins. Similarly, baroreceptor reflexes, reflexes associated with exercise, and effects of some cardiovascular disease also operate against this background. Although modification of the SkBF-internal temperature slope is occasionally seen, the most consistent effect of these nonthermoregulatory factors is to elevate the threshold internal temperature for cutaneous vasodilation. The consequence of this modification of thermoregulatory control of SkBF is that temperature regulation will often suffer when increases in SkBF are delayed or limited. Blood flow to other regions, possibly including active skeletal muscle, may also

be compromised when thermoregulatory demands for SkBF are high.

The mechanism of heat generation during dialysis is complex but at least in part involves complement activation or cytokine generation. Data on influence of dialysis on energy expenditure is scarce. Resting energy expenditure during bicarbonate dialysis of 8 W magnitude. A 10% of rise in REE could be due to absorption of glucose. In our study water quality was excellent and biocompatible membranes were used. Alteration in temperature set point due to removal of uremic toxins and variations in normal temperature rhythm of individuals are factors that could influence variations in core temperature during dialysis. Schneeweiss et al [239] did not observe an increase in REE during HD although metabolic measurements were not performed during dialysis procedure itself. Reports on energy transfer in the literature are scarce and conflicting. Provenzano et al observed net energy load from ECC to patient at dialysate temperature of 37 C at low blood flow rates . [240] The use of acetate could partly explain peripheral vasodilatation mediated heat gain. Van Der Sande et al [237] and Schneditz et al [234] observed heat loss from ECC to patient at dialysate temp 37.3 at blood flow rates of 250ml/min. [241] Our study are consistent with these data although the amount of energy transfer from patient to ECC is slightly lower at temperature of 37.3 C. The use of higher blood flow rates and differences in predialytic temperature could explain the differences seen.

Vascular response was significant during later stages of ultrafiltration and dialysis and more distinct at cooler temperatures and at lower blood volumes. The presence of vasoactive medications may have modified the response in our subjects. Better haemodynamic stability is seen when significant net heat loss is achieved. This is mainly mediated through lower core temperatures and SVR leading to indirect circulating blood volume preservation.

In conclusion the thermal balance depends on interaction of patient related factors (predialysis temperature , skin temperature, vascular tone) and dialysis factors (blood flow rates, dialysate temperature, ultrafiltration). At blood flow rates of between 350 – 550 ml/min, dialysate temperatures of below 36 C are required to achieve significant cooling. Standard temperature dialysis 37 – 37.3 C may be associated with minimal heat transfer and a rise in core temperature. The removal of heat by the extracorporeal circuit and activation of autoregulatory mechanisms to preserve core temperature may be responsible for the beneficial haemodynamic effect of lower dialysate temperatures.

Table 1 Parameters at baseline prior to dialysis

	Pre weight kg	Qb ml/min	AH drugs
Subject 1 (warm)	63.8	350	1
Subject 1 (cool)	63.4	350	1
Subject 2 (warm)	65.5	450	1
Subject 2 (cool)	65.2	450	1
Subject 3 (warm)	68	550	2
Subject 3 (cool)	67.5	550	2
Subject 4 (warm)	56.5	350	1
Subject 4 (cool)	56.3	350	1
Subject 5 (warm)	76.4	400	2
Subject 5 (cool)	76.2	400	2

AH = antihypertensive drugs

Table 2 Core temperature changes during the warm and cool dialysis

	Cool	Warm	p
Pre dialytic core T (C)	36.64 (0.33)	36.30 (0.29)	0.2
Postdialytic core temp C	36.50 (0.36)	37.12 (0.32)	0.06
Delta core temp C	-0.14 (0.16)	0.82 (0.42)	<i>0.01</i>

**Table 3a Haemodynamic and thermal parameters during
Pre UF isovolemic period at the start of dialysis .**

	Cool dialysis		Warm dialysis	
	mean	SD	mean	SD
T venous C **	35.23	0.3	37.31	0.2
Skin temp C	33.8	1.1	34.4	0.35
Cardiac output l/min	8.1	2.5	7.72	1.5
SVR dynes_cm	899	379	1154	291
RBV %	99.15	1.1	100	0.7
BP Systolic mm Hg *	155	11	128	19
BP diastolic mm Hg *	88	8	63	17
MAP mm Hg *	105	8.3	84.8	17
Energy transfer rate W *	-28.4	13.3	0.72	1.3
Duration secs	1415	185	1324	201

* p<0.05 ** p<0.01

**Table 3b Comparing haemodynamic parameters (SV,CO,RBV) between three different
blood pressure ranges recorded during all the sessions studied**

group	Systolic BP	MAP	CO	SV	RBV
1	< 100	69 \pm 2.1**	4 \pm 0.63**	85 \pm 10.6*	87 \pm 10.1*
2	100 – 125	86 \pm 0.7	8 \pm 2.6	117 \pm 28.9	95 \pm 8.3
3	125 – 150	87 \pm 3.5	7 \pm 3.1	112 \pm 47.3	99 \pm 2.2

• * P < 0.05 ** P < 0.001 (1 v 3)

Table 4 Comparing cool and warm dialysis with intermittent UF

	Prescribed Cool HD		Prescribed Warm HD		P value
	Mean	SD	Mean	SD	
Systolic BP mmHg	137.5	16.9	120.8	12.3	0.000
Diastolic BP mmHg	73.7	11.7	64.8	9.6	0.000
Hypotensive episode	2	-	5	-	-
MAP mmHg	95	12.6	83.4	9.8	0.000
Tart C	36.5	.28	37.1	.14	0.000
Tven C	35.3	.31	37.4	.17	0.000
Energy transfer Jq (kJ)	-85.3	27.2	-2.2	9.4	0.000
Heat flow rate W	-24.6	8.41	-0.62	2.78	0.000
T_Skin C	33.7	.83	34.8	.44	0.000
Core-skin C	2.8	.75	2.2	.33	0.000
Qb ml/min	387.8	94.6	410.5	78.4	0.03
CO L/min	7.2	2.1	7.6	2.8	0.24
SI l/min/m2	117.7	37.4	116.4	45.4	0.79
EDV mls	178	57.1	181.3	66.1	0.65
RBV (%)	92.1	6.4	91.3	5	0.32
SVR dynes.cm	1173.1	461.4	1098.4	523.7	0.25
UFV mls	1307	927.2	1452	825.6	0.17
Estimated REE (W)	67.7	10.1	Range (54.4 – 80.5)		

Valid complete set of observations HD cool n=172 ; HD warm n=117

Table 5 Correlations (R) of skin temperature and skin core temperature gradient with haemodynamic parameters

Parameters	T_skin (R)	Core_skin gradient
Systolic BP	-.47**	.33**
MAP	-.48**	.33**
CO	.20*	-.26*
RBV	-.04	.04
SVR	-.34**	.36**

**** P value <0.001**

*** P value <0.01**

Figure 1 Heat gain during dialysis leading to hypotension
Vasoconstriction or heat transfer from the dialysis machine or both?

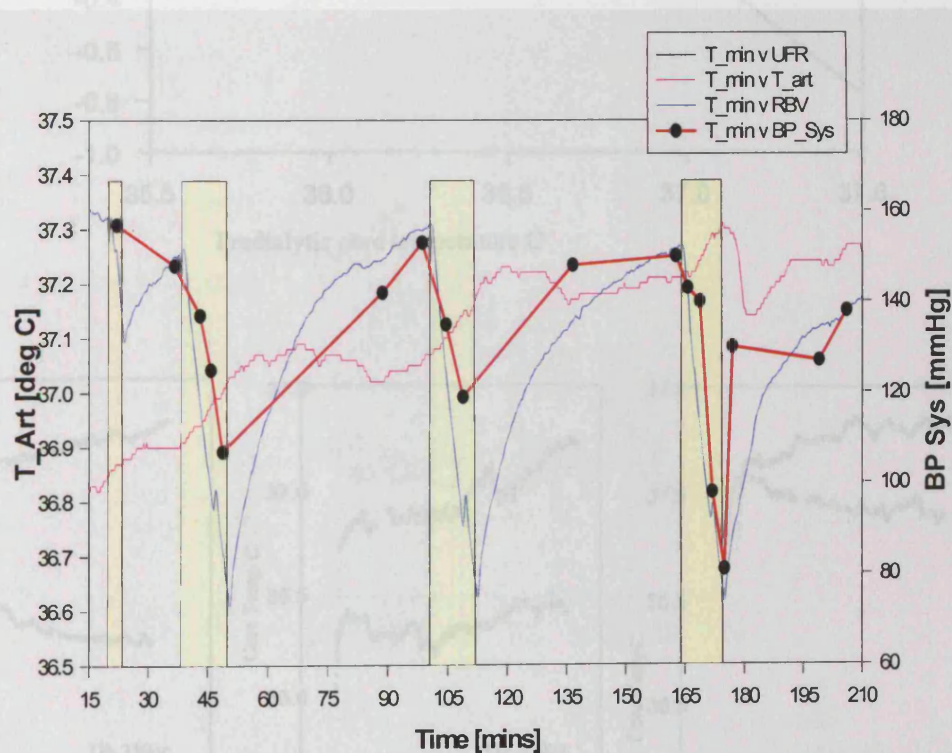
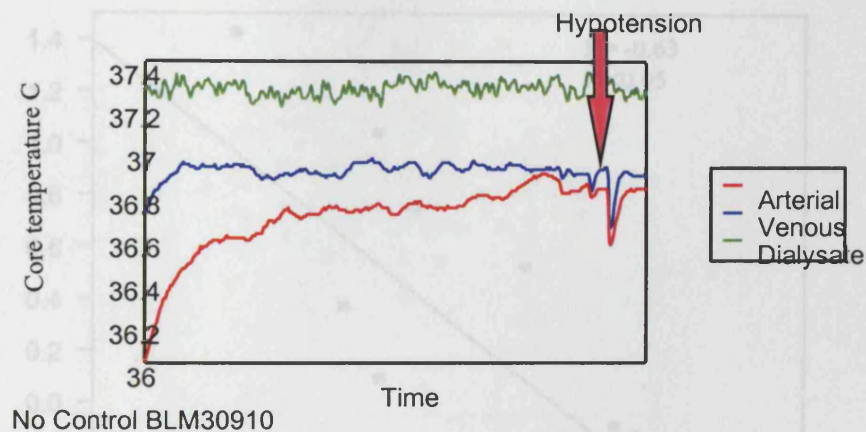


Figure 2 Core Temperature rise during intermittent ultrafiltration steps in a single patient caused by vasoconstriction

Figure 3 Relation between heat gain (change in core temperature) and predialytic core temperature in all treatments

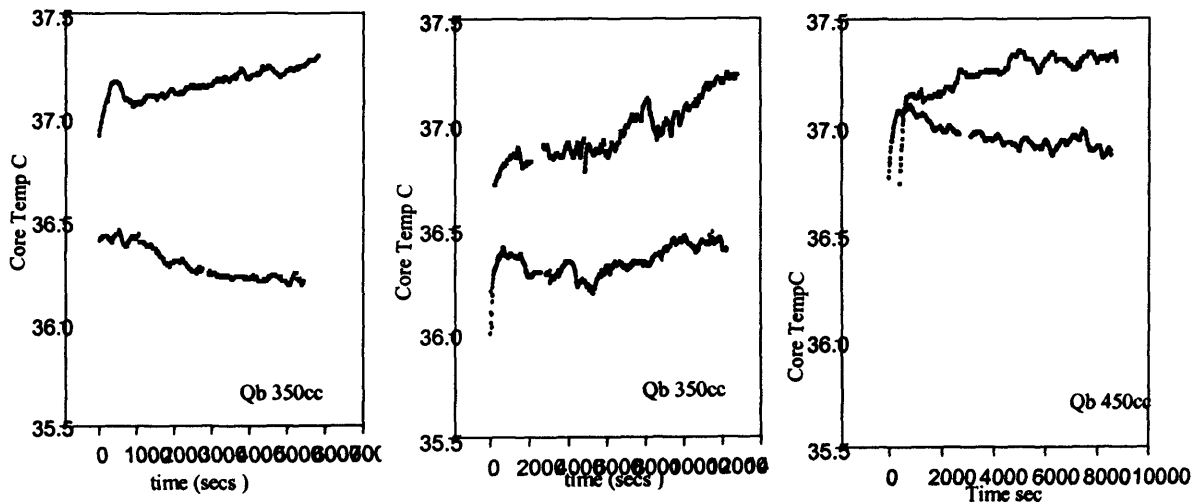
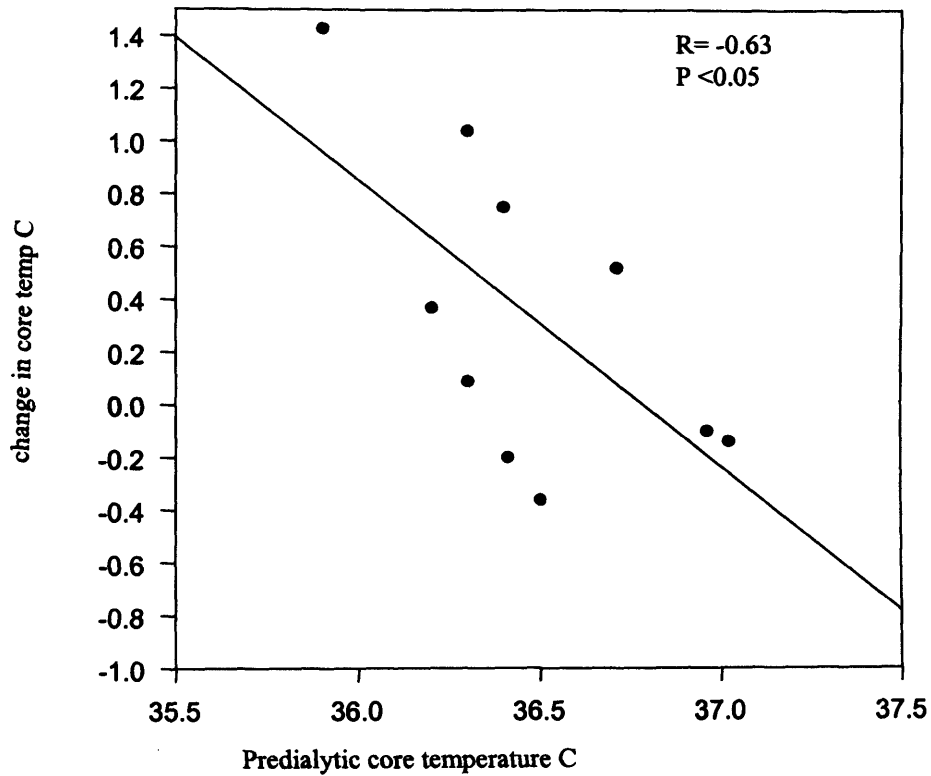


Figure 4 Core temperature profile during dialysis in 3 subjects with paired warm and cool dialysis

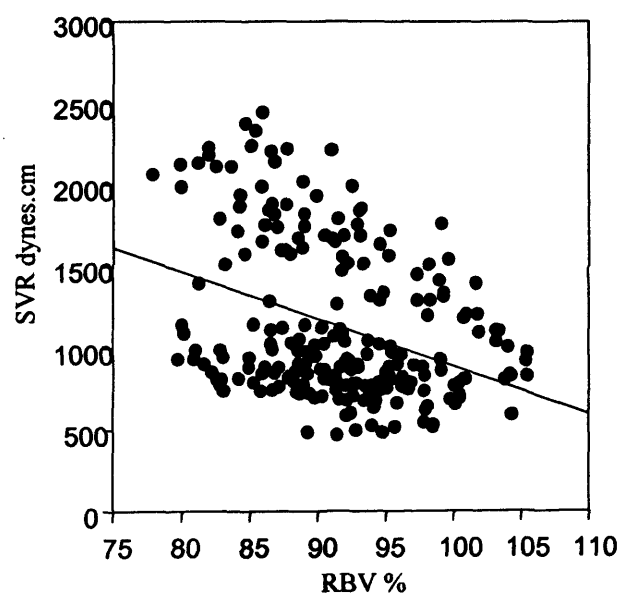
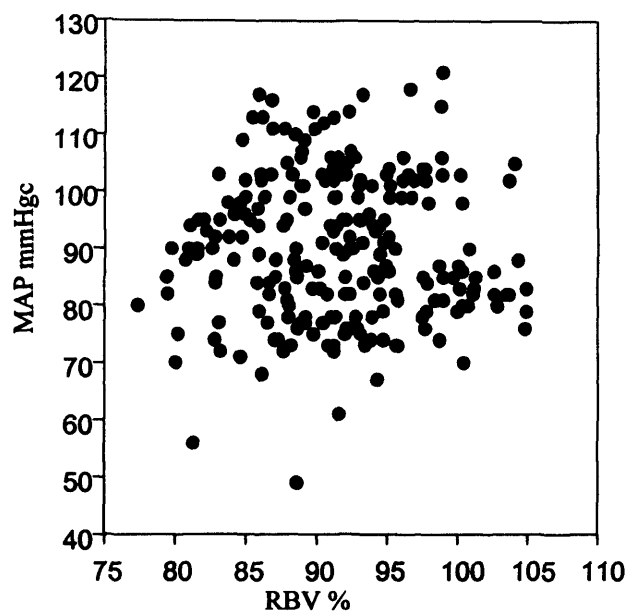


Figure 5 Relationship of RBV with MAP (p=ns) and calculated SVR ($P < 0.01$, $R = -0.34$)

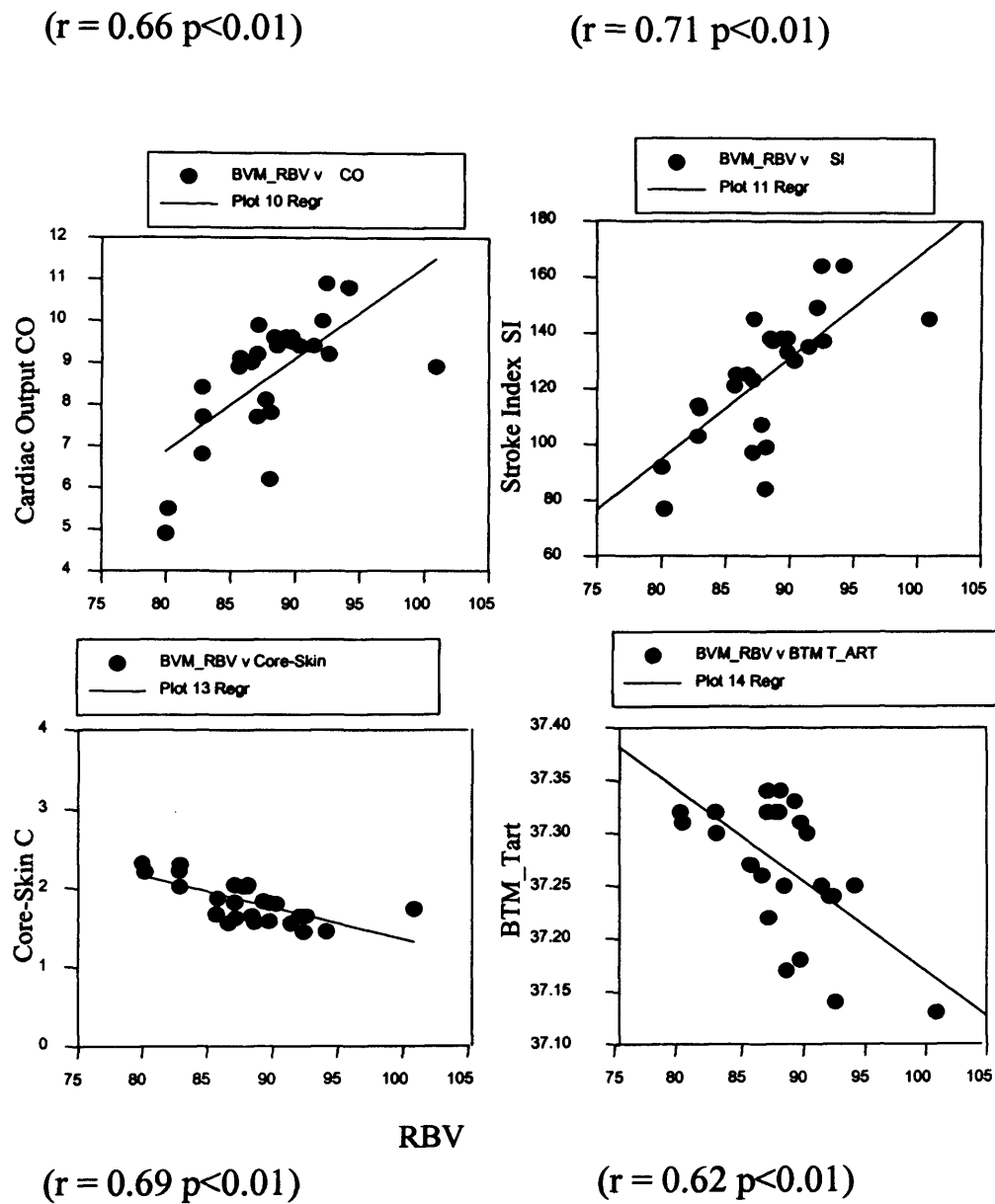


Figure 6 Relationship of relative blood volume to haemodynamic and thermal variables (in a single study Td 37.4)

Figure 7 Energy transfer at different blood flow rates during warm Td 37.3C (7a) and cool dialysis Td 35.3C (7b)

Fig 7a

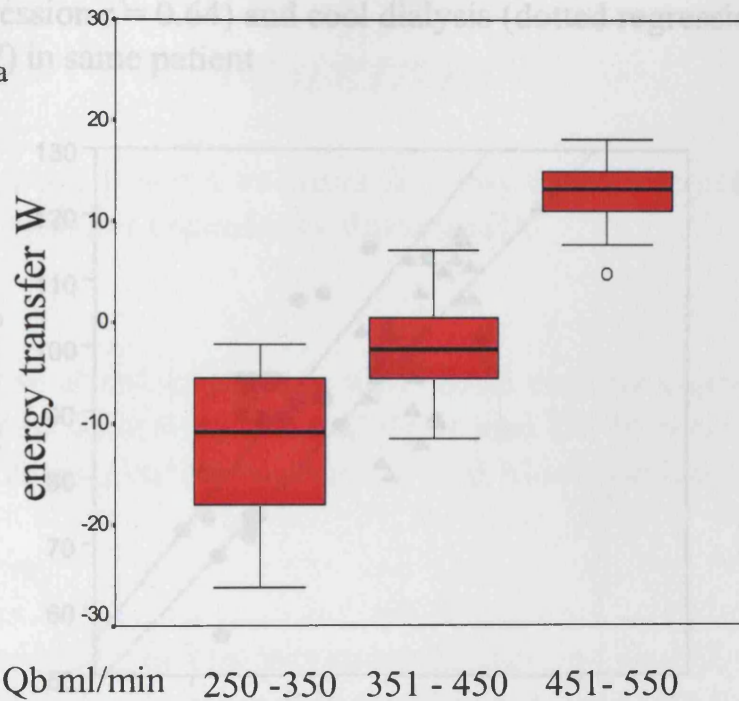


Fig 7b

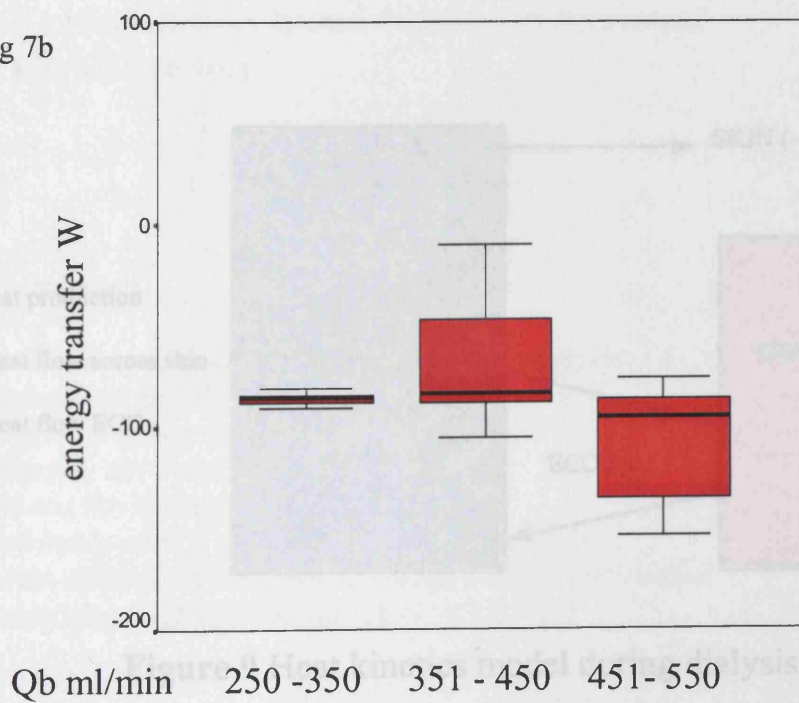


Figure 8 Significant positive correlation between MAP and Core-skin temperature gradient both for warm (bold linear regression $r = 0.64$) and cool dialysis (dotted regression $r = 0.57$) in same patient

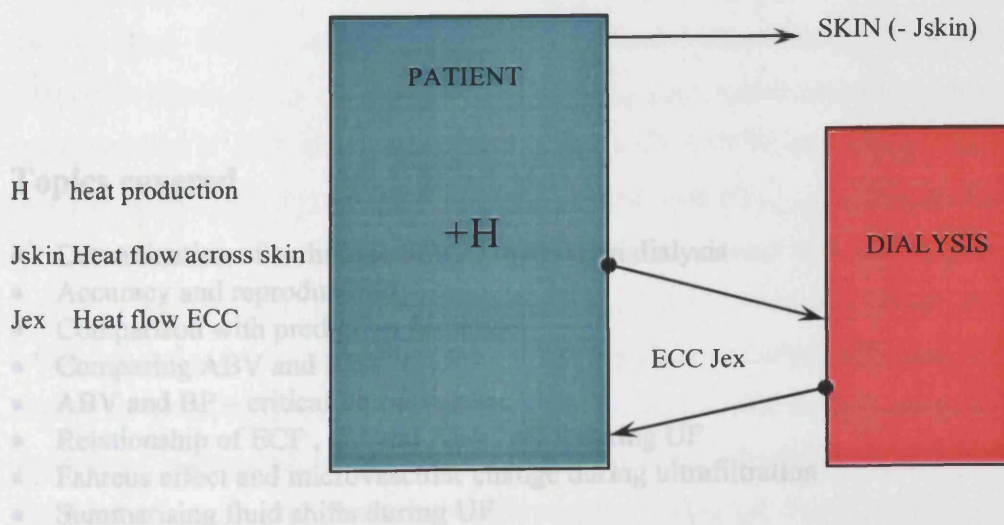
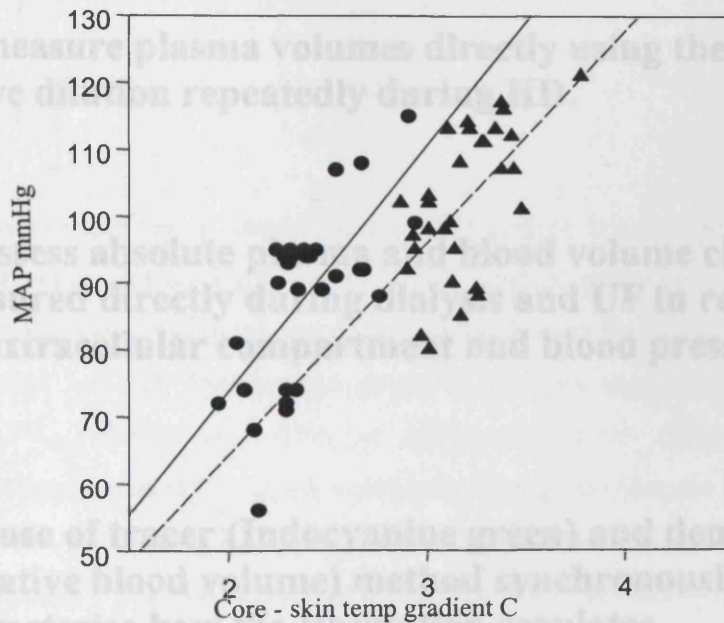


Figure 9 Heat kinetics model during dialysis
(+ indicates net heat gain, - indicates coolin)

Section on absolute blood volume studies

OBJECTIVES

- **To measure plasma volumes directly using the principle of dye dilution repeatedly during HD.**
- **To assess absolute plasma and blood volume changes measured directly during dialysis and UF in relation to the extracellular compartment and blood pressure**
- **The use of tracer (Indocyanine green) and density (Relative blood volume) method synchronously to characterise how the circulation regulates and redistributes blood volume in response to ultrafiltration**

Topics covered

- Determination of technique of ICG method on dialysis
- Accuracy and reproducibility
- Comparison with prediction formulae
- Comparing ABV and RBV
- ABV and BP – critical blood volume
- Relationship of ECF , BP and ABV, refill during UF
- Fahreus effect and microvascular change during ultrafiltration
- Summarising fluid shifts during UF

Chapter 7

Serial determination of absolute plasma volume during haemodialysis using indocyanine green

Summary

Haemodynamic stability in haemodialysis (HD) largely depends on plasma volume (PV) preservation during ultrafiltration (UF). Current estimates of blood volume (BV) are indirect or involve the use of radioactive tracers that do not allow repeated measurements on HD. Indocyanine green (ICG) was used to measure plasma volumes (PV_{icg}) during HD. After an initial pilot phase (Phase I), PV were determined predialysis (PV_{icg_preHD}), repeatedly during isovolaemic HD (PV_{icg_isoHD}) (Phase II) and during step UF (Phase III). Absolute blood volumes (ABV_{icg}) were calculated using PV_{icg} and haematocrit. Patients were monitored for extracellular fluid (ECF bioimpedance) and relative BV changes (ultrasonic). Phase I demonstrated stability of the dye in plasma, peak absorbance at 805nm and a short half-life (4.53 ± 1.5 min). 10 mg dye (2.5mg/ml) was injected for each PV_{icg} measurement. Eight plasma samples were obtained 3-min after injection at 1-min interval to obtain decay characteristics. The PV_{icg_isoHD} measurements had excellent reproducibility (r^2 0.98 method SD 356 mls, mean CV 4.07 %) and a mean difference (MD) \pm SD of 149 ± 341 mls only when compared with PV_{icg_preHD} (Bland-Altman). PV_{icg} at the beginning of dialysis was significantly correlated with the body surface area (r^2 0.82, $p < 0.001$) and to ECF estimates (r^2 0.73, $p < 0.001$). Blood volumes calculated using prediction formulae significantly underestimated ABV_{icg} at the start of dialysis ($p < 0.0001$). The findings here demonstrate that this method can be used to determine PV repeatedly during HD with excellent reproducibility. It is a potential tool for further research in haemodynamic stability during UF.

Introduction

Hypovolemia plays an important role in symptomatic hypotension, which complicates upto 25 % of dialysis treatments. This has generated considerable interest in blood volume (BV) determinations during ultrafiltration (UF). Attempts to quantify absolute BV during haemodialysis have been limited by lack of a suitable method. Methods involving radioactive tracers are unsuitable for routine clinical use and do not allow repeated measurements. [242] In vitro studies have revealed a possible mutagenic potential for Evan's Blue [243]. Thus current estimates of blood volume during dialysis are indirect or provide relative changes only and have only been validated against anthropometric data derived from the normal population [212] or a single predialysis measurement. In 1968 Bradley and Barr reported on BV measurements using indocyanine green (ICG) in a limited number of patients [244]. This method has since been used for estimation of liver blood flow and cardiac output measurements but has not been studied during haemodialysis. The in-vivo properties of this tricarboyanine dye allow its repeated use within short interval [153]. This study examined the feasibility of using the dye for repeated PV determination in haemodialysis. I wished to a) determine the technique of PV determination during haemodialysis using AV fistulae and to determine its reproducibility b) observe changes in PV during UF on dialysis and c) compare BV with standard prediction formulae. In this chapter experiences with this method and some results of its practical application have been described.

Methods & protocol

Study Design : The study was performed in three phases.

Phase I (5 studies)

This pilot phase was performed to determine the method, ascertain dose, dye kinetics, sampling and calibration techniques in haemodialysis patients. Technique for the use of ICG using both the fistula and the extracorporeal circuit was optimised, the quality of which may be depicted from the graphs below. Determination of minimum dose, injection site in the extracorporeal circuit, half life determination,

assay variability, absorption characteristics of the dye, possible estimation errors and technique artefacts were individually studied and resolved. The errors during sampling have been minimised and the assumption of 1st order kinetics of ICG decay may be verified from the linearity of the log plot. Fig 1a

Dialysate samples were collected from the dialyser outlet port to look for ICG absorbance immediately and 3 mins after dye injection to detect any leakage of dye across the dialyser.

Phase II (9 studies)

The purpose of this phase was to test reproducibility and method variation. Plasma volumes were measured with patient supine for 20 min just before dialysis (via fistula needles) followed by triplicate measurements at 20 min intervals during an isovolemic period in the first hour of dialysis via sample port. Fig 1b

Phase III (10 studies)

Plasma volumes were determined directly during dialysis with ultrafiltration (3l/hr) using 4 intermittent UF steps (removing 40-20-20-20% of total UF volume) and intervening rest periods (Fig 1c), with BV measurements at the beginning and the end of first and fourth ultrafiltration boli during steady state conditions. (Fig 1d). All measurements and ultrafiltration commenced after an equilibration period of twenty minutes from the connection to the extracorporeal circuit.

Subjects

The North Herts Ethical Review committee approved the study. All patients gave informed consent. 24 studies were performed during routine dialysis sessions on 17 subjects (4 females) of different body sizes and a wide range of interdialytic weight gains (Table 1). Subjects had been receiving chronic haemodialysis for at least 6 months with a stable dry weight and normal liver function tests. The presence of iodine allergy, eosinophilia, raised serum IgE levels or significant access recirculation within one month prior to the study were exclusion criteria. All patients were on thrice-weekly high flux dialysis (Fresenius 4008E) using AV fistula. (Blood flow rates were in the range of 350–450 ml/min and the mean weekly Kt/V was 1.24 +/- 0.16) [112] 15 patients failed selection and screening criteria.

Tracer

The tracer used was Cardiogreen (ICG Green TM Sterile indocyanine green USP Fluka), a tricarboyanine dye, mol wt 775, with an absorption peak at 805nm (Fig 1e). The dye is nontoxic, confined to plasma, not subject to extravascular distribution, and not metabolised or degraded. Following injection, the dye is rapidly bound to plasma proteins. After equilibration, the dye decays fast in an exponential manner. It is exclusively taken up by the liver and has a plasma half-life 2-3 min (the time required for the initial concentration of the dye to be halved). [152] The elimination characteristics resemble the Michaelis-Menten kinetics [153]. Michaelis-Menten kinetics refers to the mechanism of ICG dye disappearance from plasma. The nature of the decay resembles Michaelis-Menten kinetics in as much as the fact that the dye disappearance rate has in a complicated way, an almost linear dependence on the concentration of the substrate at low concentrations. The decay occurs at approximately 18 – 24% of the dye concentration/min (K_{icg}) and is therefore a function of the dye concentration. [153]

Calibration

Calibration was performed for each blood volume measurement using the blank plasma sample just before dye injection.

5-point calibration (Fig 1f)

1. 30mls of distilled water were added to 1ml of neat ICG solution (2.5 mg/ml) to prepare a dilute calibration fluid. Syringes were weighed before and after the contents had been added to obtain the exact volumes.
2. A microcuvette was placed in the spectrophotometer (Phillips 8700 series) and zeroed using a fixed wavelength of 805 nm. 1ml baseline plasma sample was injected into microcuvette and re-weighed to obtain exact plasma volume. The absorbance of the blank plasma was then determined.
3. 4 X 10 uL aliquots (10 uL micropipettes CV <0.5%) of dilute calibration fluid were added incrementally to the plasma in the cuvette and its absorbance measured at each incremental step. The mean of four readings was taken at each step, the cuvette being removed, agitated and replaced between measurements. The dilution effect of adding the aliquot volumes was taken into account to improve precision. After 4 x 20 uL aliquots have been added, the concentration

of dye in the plasma would have been only 3.8% lower than the real value if the dilution effect had not been considered.

2-point calibration

A concentrated calibration fluid was prepared using 1 ml ICG (2.5mg/ml) added to 7 ml distilled water. 10 uL of fluid was added to a known volume of blank plasma and the absorbance determined.

Procedure for direct determination of plasma volume and derived absolute blood volumes

Prior to each dye injection, blood was withdrawn for Hct and baseline plasma blank in heparinised syringes. ICG (25 mg) was dissolved in 10 ml of sterile aqueous solvent to produce ICG solution of 2.5mg/ml. 10 mg of dye was then injected as rapidly as possible into a venous port beyond the bubble trap [152]. All syringes were weighed on a precision scale before and after the injection to determine the precise quantity infused. A comparison of the injected ICG amounts as weighed or read from the syringe marks, respectively showed that about 99% (+/- 1.1%) of the cited amount were injected. Exactly 3 min after the end of the injection, sampling was commenced from the arterial port in heparinised syringes at 1-min intervals for 10 min (8 samples). Samples were centrifuged at 3000 rpm for 10 minutes. The plasma blank sample was used to determine the baseline background absorbtion at 805-nm wavelength. The absorbtion of ICG dye in the timed plasma samples were then compared against the baseline at the same wavelength [245]. Only 500 uL of plasma was required from each spun sample due to the use of half microcuvettes. Each ICG BV assay in the lab for an individual patient study in Phase II required approximately 4 hrs. Phase III therefore required very intensive work, each study involving approximately 9-10 hrs of bedside and laboratory work.

Other techniques

Patients were monitored continuously for blood pressure (Oscillometric) BPS08, Fresenius Medical Care, relative BV changes (ultrasonic BV monitor, Fresenius) [124] and extracellular volume estimates (multifrequency Whole Body Bioimpedance, Hydra, Xitron Technologies). All these data were collected using a

data acquisition software (Acqi, Fresenius). Haematocrit was measured using the Coulter Counter (STKS) method. During these studies, ultrafiltration volume was verified by collecting ultrafiltrate in a measuring cylinder.

The data quality in some of the studies have not been satisfactory due to various technical problems. The data from two studies had to be discarded due to a problem with the data acquisition.

Analysis

The natural logarithm of the measured ICG dye concentrations were plotted against time for each plasma volume measurement and the best fit linear regression obtained from the data points. Fig 2 depicts such a concentration-time curve (\ln ICG v time) and its linear regression confirming excellent linearity of the data. Natural logarithm is inverse of an exponential function [e.g. $\text{EXP}\{\text{LN}(4)\}=4$]. Therefore the obtained linearity confirms the exponential nature of the dye concentration decay. This is in agreement with the description of the dye pharmacokinetics in the literature. The extrapolation of the straight line backward allows determination of the initial logarithmic dye concentration (offset from the regression line). The antilog of the offset yields the initial dye concentration in plasma at the time of injection. Plasma volume (PV) was calculated according to the Eqn 1 (Appendix).

BV was derived by using the Hct adjusted for by a factor of 0.86 to correct for a) the difference between the Hct in the systemic circulation and whole body Hct (F cell ratio 0.90) and b) trapped plasma (~ 4%) [$\text{Hct}_{\text{body}} = 0.90 \cdot 0.96 \cdot \text{Hct}_{\text{sys}} = 0.86 \text{ Hct}_{\text{sys}}$]. (Eqn 2. Appendix). PV measured during dialysis was compared to predialysis measurements after correcting the former for the volume of the extracorporeal circuit (internal fibre volume measured predialysis by Renatron analyser and volume of the circuit measured using saline). PV readings during the isovolemic phase were corrected for changes in plasma protein concentration that was observed on the relative blood volume monitor (mean variation of 1.2%), to correct for any PV shifts induced by osmolar variation [245]. Statistical methods used were Bland Altman [246] analysis required for comparison of methods, t test for comparison of means ($p < 0.05$), linear regression and Pearson correlation tests. Statistical analysis was

performed using software package Sigmaplot (version 2.01) and curve fitting software (Table Curve 2D).

Results

Calibration results

There were marked difference between the absorption slopes obtained during the 2 and 5-point calibration procedure (Fig 3). The two-point calibration appeared to consistently overestimate the slope in the 3 patients studied. The difference is possibly due to the assumed zero readings in the 2-point calibration. For this reason, the 5-point calibration technique was deemed more precise and was used for phase II and phase III studies. Four 5 point calibration curves were obtained for all 19 studies performed in phases II and III (76 calibrations). The slopes obtained were highly consistent with no significant intraindividual variation. However, the background absorbance (intercept on the absorbance axis) varied considerably (Table 2).

Phase I

The use of a 5 mg bolus of ICG resulted in very low concentrations of ICG in the plasma particularly in the tail of the dilution curve. Lack of precision in this area led to consistent over estimation of absolute PV. Administration of at least 10 mg ICG (2.5 mg/ml), as used for most previous studies [152], was necessary to avoid this potential source of error. A site of injection close to the venous needle site was required as injection into the bubble trap induced a time delay on the decay curve. The problem was circumvented by use of venous sample port, immediately adjacent to the venous needle site. Peak spectral response was at 805 nm in plasma, blood or haemolysed blood (by repeated laboratory wavelength scans). Spectral stabilisation was sufficiently rapid for measurement of PV and BV and was very reproducible. The clarity of the plasma extracted from the blood sample is crucial. Significant interference was observed with hyperlipidemic, immediately postprandial and grossly haemolysed samples in this pilot phase. To determine the most appropriate sampling interval, the standard deviation of measurements using different sampling time was calculated from the regression mean. This confirmed that sampling commencing at 3 or 4 min following the ICG infusion led to the most consistent

estimations. One subject, elderly with congestive cardiac failure and atrial fibrillation, did not show complete mixing after 6 min.

Samples were stable for approx. 8 hrs when stored at 4°C. Use of heparin did not affect the absorption spectra. There was no absorbance detected at 805 nm in the dialysate aliquots taken immediately after the infusion of dye. This confirmed that the dye was suitable for use during haemodialysis.

Predialysis compared with isovolemic dialysis

Mean PV measurements during isovolemic dialysis compared well with measurements just before dialysis with an acceptable mean difference of only 149 ± 341 mls between the predialysis and the first plasma volume measurement (PV1) during isovolemic dialysis. (Fig 4)

Reproducibility during isovolemic dialysis (Phase II)

Three PV measurements during the first hour of isovolemic dialysis were consistent and highly reproducible. Correlation coefficients between measurements PV1 & PV2 ($R^2 = 0.98$) were significant. As a measure of repeatability the mean differences (MD) of the first and second measurements ($MD = PV2 - PV1$) and the SD of differences were calculated as mean difference of 33 ± 128 mls. The method mean SD was 356 mls and mean CV 4.07 %. There were no traces of ICG in the baseline blank plasma on repeated measurements. Mean half-life of dye was 4.53 ± 1.5 min.

Measurements during ultrafiltration (Phase III)

The ICG decay parameters during this phase of the experiment were consistent with previous studies (Table 2b). There was a significant reduction in PV detectable in all subjects during ultrafiltration except in patient 2, who did not tolerate the fourth UF boli and required saline infusion. The method was sensitive enough to detect this [Table 3]. Mean arterial pressure was significantly correlated with directly measured circulating plasma volume ($r=0.70$, $p<0.01$). There were no adverse reactions to the dye.

Comparison with prediction formulae

Measured PV during the initial isovolemic period in both phase II and III significantly correlated with extracellular fluid volumes measured by bioimpedance in 14 patients $r^2 = 0.73$ (Fig 6). Reliable estimates of ECF could not be obtained in five patients in phase II and III. The PV directly obtained also correlated significantly with body surface area (Eqn 3 Appendix and fig 7). The predicted BV using weight and height formulae, however significantly underestimated ($p < 0.0001$) the ICG measured absolute BV values. Regression lines for the Guyton, Hidalgo, Allen and Baker method [247,248,249,250,251] (Eqn 4,5,6,7 Appendix) showed a wide scatter with a mean difference (\pm std deviation) of -0.96 (± 1.2), -1.6 (± 1.7), -1.2 (± 1.4) and -1.4 (± 1.7) litres respectively.

Discussion

Tracer methods for determination of plasma and blood volumes have a number of drawbacks including the need for special equipment and often the requirement for the use of radioactive substances. The ICG method proposed by Bradley and Barr did not initially gain widespread acceptance, probably due to their use of a non-standard device designed for cardiac output measurement. The spectrophotometer method was first described by Schad et al (1987) in dogs' [252]. The method required the use of central venous injection and sampling. The presence of an extracorporeal circuit and of high flow fistulae in haemodialysis patients greatly facilitate the use of this method for serial BV determination during haemodialysis, obviating the problem of central vein sampling. The favourable qualities of ICG are well documented and spectrophotometer equipment is widely available. The distribution space of ICG is similar to the plasma volume. Estimates of plasma volume obtained using this method have been shown to correlate well with estimates compared to isotopic and Evans Blue methods. [152] There are very few reported adverse events. [253] The half-life of the dye in this study corresponds well with the data from the literature. [254]

There are some methodological limitations of this technique. Since the half-life is short compared to other tracers, accurate timing of the samples is critical. On the

other hand the short half-life makes ICG a suitable tracer for repeated measurements at short intervals without the disadvantage of dye accumulation as we have shown in this study. A general drawback is the need for a calibration curve for each patient and intraindividually varying conditions during HD. 5-point calibration was performed to be sure that the relationship between absorbance and ICG concentration was linear, cross check for an errors introduced by addition of ICG aliquots into plasma blank, determine any offset that may have been present and eliminate any ambiguity which might arise in assuming that if the background absorbance is close to zero in one patient this result may be extrapolated to all patients.

This experiment demonstrated that with precise 5-point calibration, consistent slopes may be obtained. The intercepts for the blank plasma samples were however significantly different presumably related to varying plasma composition during dialysis. This finding suggests that a single 5-point calibration per patient studied is sufficient, provided the baseline absorbance (intercept on absorbance axis) is determined before each measurement. The slight variation between predialysis and isovolemic dialysis readings in phase II could have been due to volume shifts induced by osmolar variation or dead space volumes in the fistula needle predialysis. The platelet count and serum albumin may affect the disappearance rate (K_{icg}). The R_{max} (maximal removal rate) largely depends on LCAT and cholinesterase [255]. This may introduce an error in hyperlipidemic samples. Dissociation between R_{max} and K_{icg} is possible in liver diseases like cirrhosis and obstructive jaundice.

Apart from these minor drawbacks, the method is highly reproducible with a variation (CV 4.07 %) well within the limits of the other tracer methods (e.g. 6.5 % for Radioimmunolabelled human serum albumin and 17% for Evans blue) [256]. The variations are likely to be due to variations in blood sampling technique, variations in stability of the physiologic parameters of the subjects, errors in the measurement of Hct, errors due to trapped plasma (3-4%) and unmeasured stroma proteins (these can cause an underestimation < 1 % or 6 ml/l blood).

Incomplete mixing can potentially introduce a significant error in the measurement. Analysis of the concentration time curve helps to identify the moment of complete mixing. During the phase 1 study I observed that prolonged mixing might occur in

presence of cardiac dysfunction, hence a wide margin is desirable from injection to sampling period. At the same time, due to rapid decay, one must avoid a situation where the measured plasma dye concentrations are small.

Although sampling can be begun at 1-1.5 min after infusion of the dye, we found 3 mins as ideal to avoid such potential mixing problems. Incomplete mixing was not encountered in phase 2 and 3 as evident from the decay curves obtained. In agreement with other studies complete mixing of dye was obtained after 3 min in all subjects in phases II and III [257]. Analysis of the logarithmic data plotted on a scale helps to identify the moment of complete mixing. Mixing time may vary especially in subjects with circulatory failure.

Indirect estimates of BV based on anthropometric data significantly underestimate directly measured BV changes, especially at the upper end of the range possibly because they are derived from databases and metanalyses obtained from normal healthy population. [258] However it has been shown here that within the haemodialysis population itself directly measured volumes showed good correlation with extracellular fluid volume (fig 6) and body surface area (fig 7). ICG derived ABV has haemodynamic significance and may play a central role in preserving vascular stability during UF. In conclusion, indocyanine green is a suitable tracer for determining plasma volume repeatedly in subjects on haemodialysis. This method provides excellent reproducibility, can be carried out in most laboratories and provide a reference method for further research in hemodynamic instability on dialysis.

Table 1 Biometric data of patients studied

	Mean	SD	Range
Age (yrs)	61.1	10.1	50-78
Dry body Weight (kg)	77.5	19.0	53.3 – 112.3
BSA (m ²)	1.91	0.27	1.54 - 2.42
Ht (cm)	168.8	8	158 - 184
IDWG (litres)	2.3	1.01	0.8 – 4.15

Ht = height ; IDWG = interdialytic weight gain

Table 2a Results of four 5-point calibrations curve in all 19 subjects in phase II and III.

Calibration	Mean Slope (SD)	Mean intercept (SD)
1	0.296 (0.013)	0.047 (0.051)
2	0.296 (0.011)	0.040 (0.050)
3	0.285 (0.017)	0.030 (0.031)
4	0.285 (0.019)	0.045 (0.045)

SD = standard deviation No significant differences between the measured calibration slopes obtained ($p>0.05$)

Table 2b ICG decay kinetics during four blood volume measurements in 10 patient during ultrafiltration (Phase III)

Blood volume Measurements	Mean Half time Mean (sd) mins	SD of log ICG Mean (sd)	Decay constant
1	4.73 (1.17)	0.26 (0.14)	-0.0038 (0.0008)
2	5.53 (1.41)	0.23(0.09)	-0.0031 (0.0006)
3	6.17 (1.51)	0.23 (0.01)	-0.0028 (0.0007)
4	6.30 (1.56)	0.23 (0.11)	-0.0028 (0.0007)

Sd = standard deviation

Table 3 Absolute blood and plasma volumes before and after first and final ultrafiltration steps in ten patients on dialysis (Phase III)

Sub	PV 1	PV 2	PV 3	PV 4	BV 1	BV 2	BV 3	BV 4	Pre Weight	Post Weight	Pre-post weight
1	5698	4818	4256	3976	8033	7139	6157	5944	110.8	107.6	3.2
2	3457	3070	2376	2626	5195	4625	3598	3977	58.8	58	0.8
3	4195	3779	3452	3160	5994	5578	5262	4881	70.6	68	2.6
4	3357	3196	2225	2077	4682	4670	3507	3269	82.1	78	4.1
5	5836	5169	4773	4562	8248	7450	6905	6717	102.7	99.8	2.9
6	6038	5200	4306	3779	7726	6849	5790	5093	85.5	82.5	3
7	4694	4467	3807	3492	7081	6854	5960	5504	73	71	2
8	4903	4484	4197	3957	7283	6791	6633	6392	85.3	82.8	2.5
9	6759	6516	6017	5716	9670	9450	8781	8363	113	111	2
10	4724	4492	4183	4134	6717	6387	6059	6018	74.8	72.3	2.5

Sub = Subjects , BV= blood volumes (mls), PV= plasma volumes (mls),
Pre weight = predialysis weight (kg) , Post weight = postdialysis weight (kg),
Pre – post weight = net weight loss during dialysis (kg)

Figure 1a The exponential disappearance of ICG (A)

The errors during sampling have been minimised and the assumption of 1st order kinetics of ICG decay may be verified from the linearity of the log plot. (B)

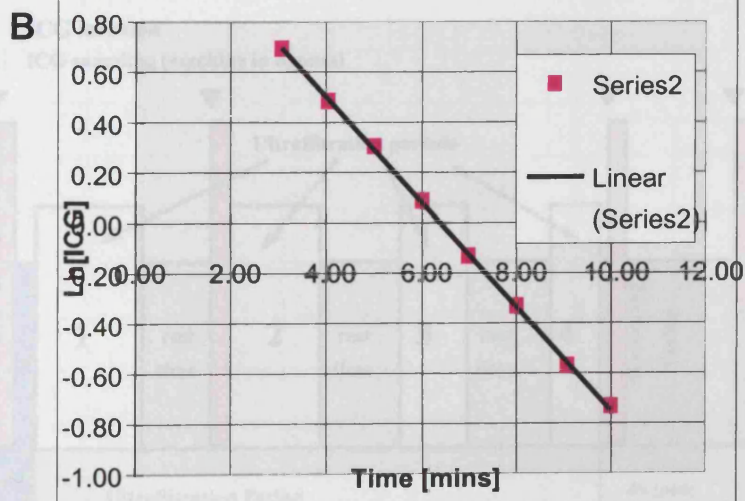
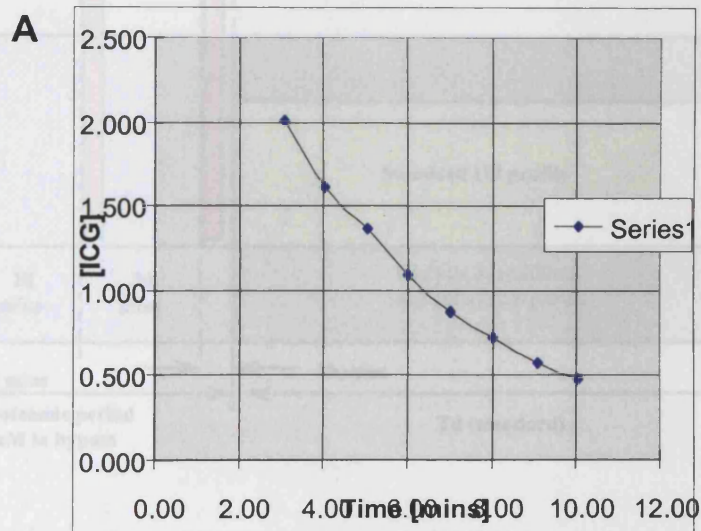


Figure 1b UF protocol for ABV reproducibility phase

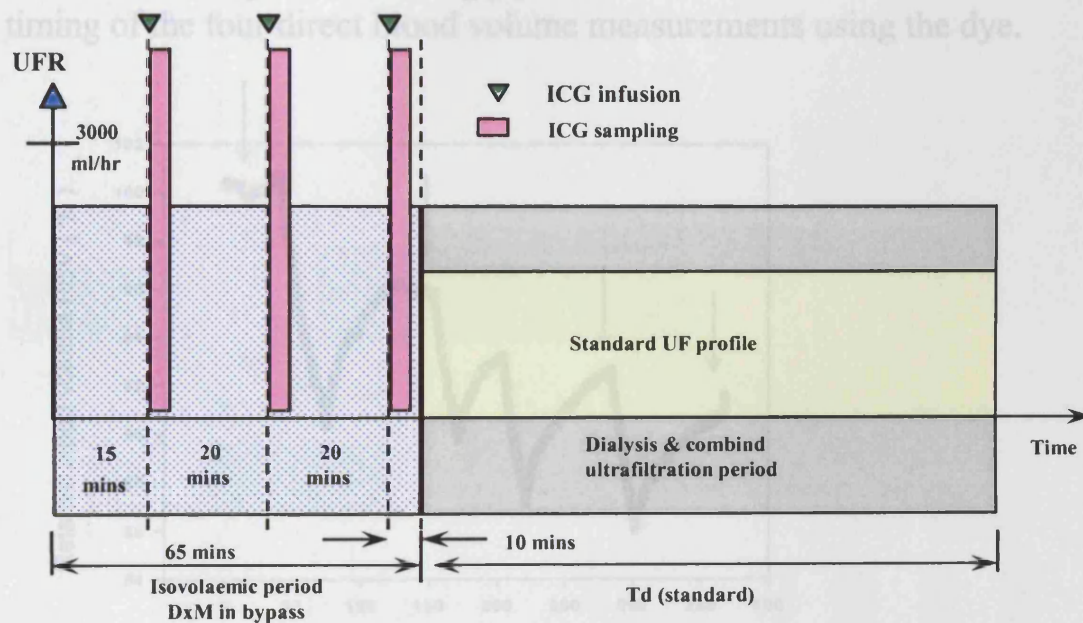


Figure 1c Protocol for ICG measurements during Phase II and phase III

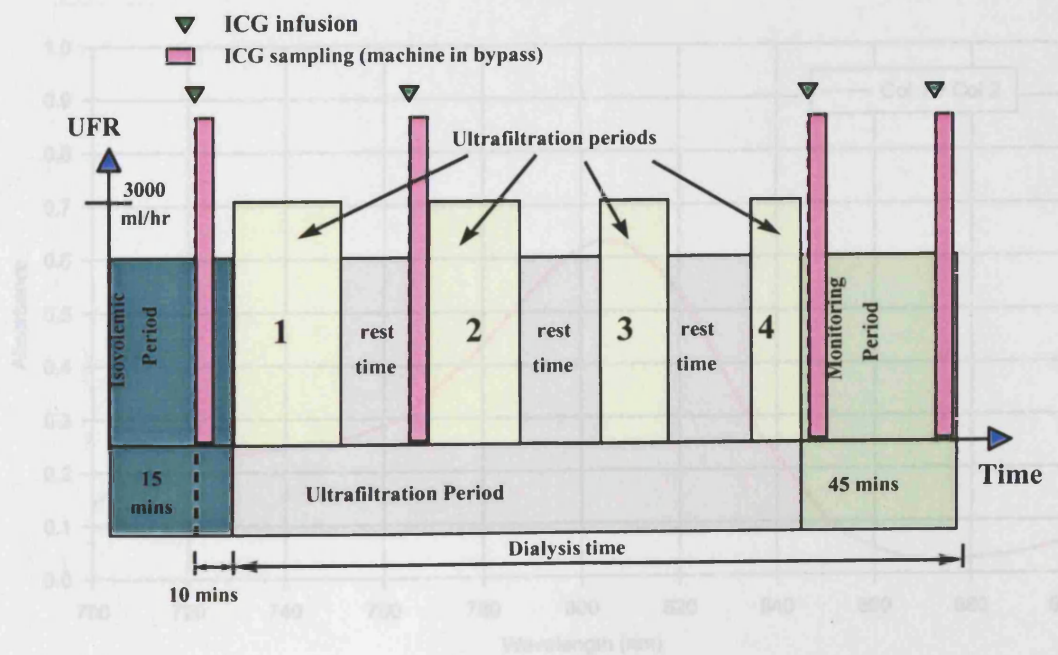


Figure 1d Relative blood volume profile during intermittent ultrafiltration in a patient during phase III. Arrows indicate the timing of the four direct blood volume measurements using the dye.

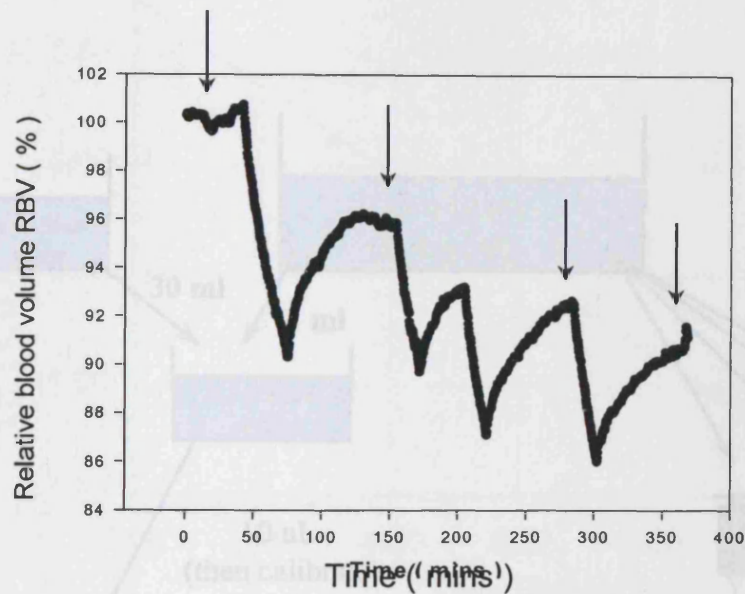


Figure 1e Spectral response of ICG peaks at 805nm.

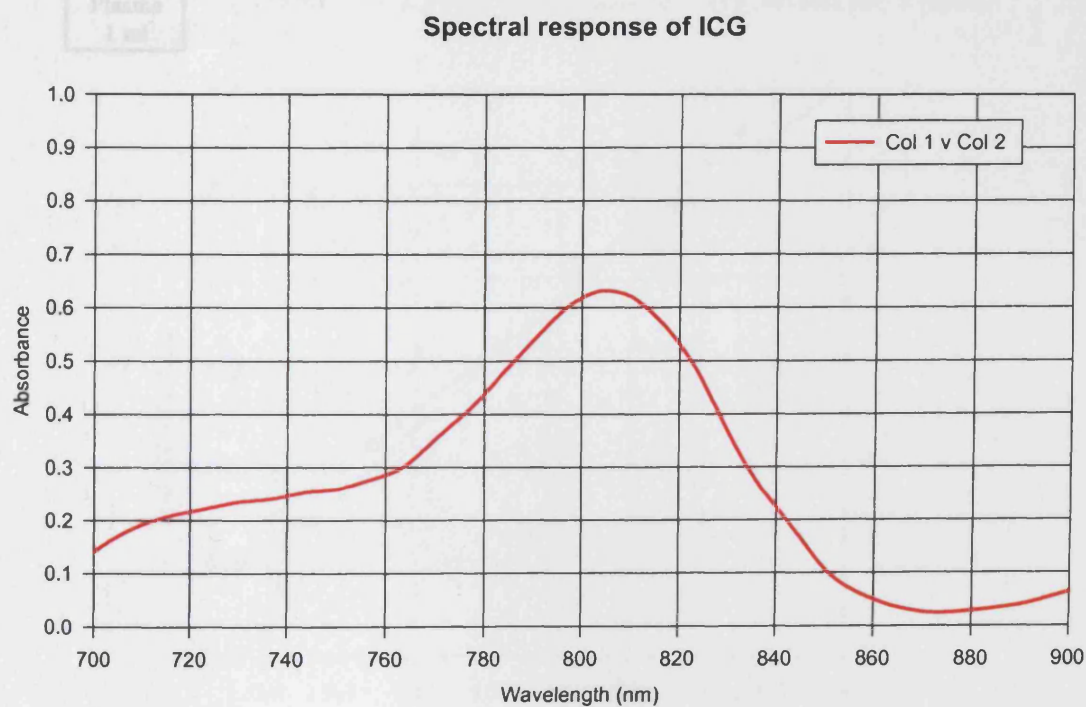


Figure 7 A concentration time plot 3-10 mins after injection of dye. Y axis represents the $\ln [ICG \text{ mg/l}]$ concentrations derived from measured absorbances and the calibration curve. The linear regression line ($y = -0.1973x + 0.7412$, $R^2 = 0.99$) extrapolated backwards to obtain the dye concentration at the time of injection.

Figure 1f Calibration experimental steps

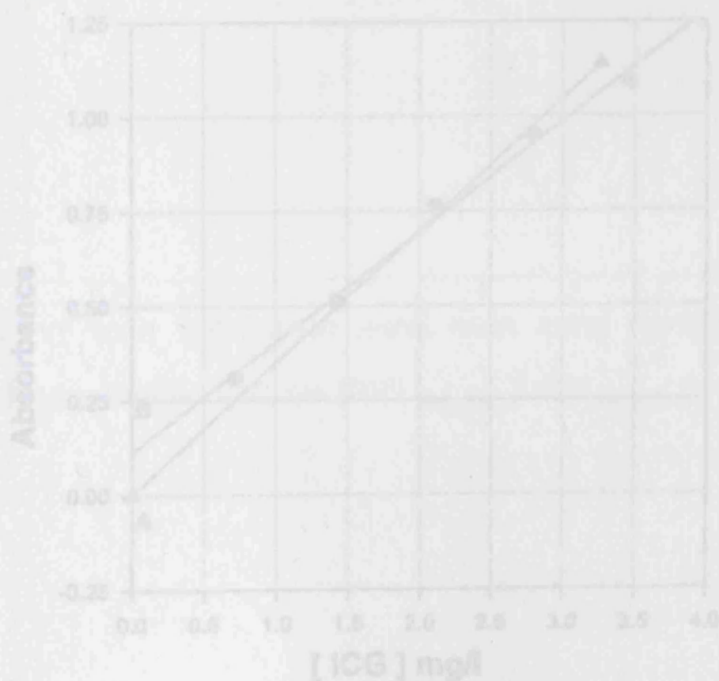
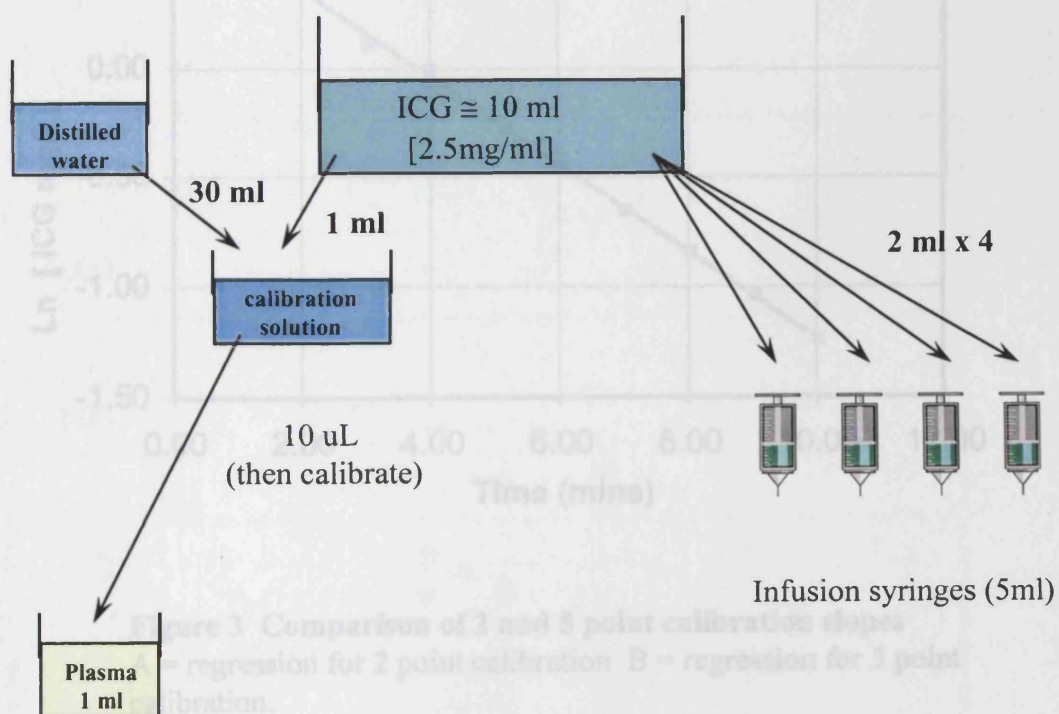


Figure 2 A concentration time plot 3-10 mins after injection of dye.
Y axis represents the Ln [ICGmg/l] concentrations derived from measured absorbances and the calibration curve. The linear regression line ($y = -0.1973x + 0.7412$ $R^2 = 0.99$) extrapolated backwards to obtain the dye concentration at the time of injection.

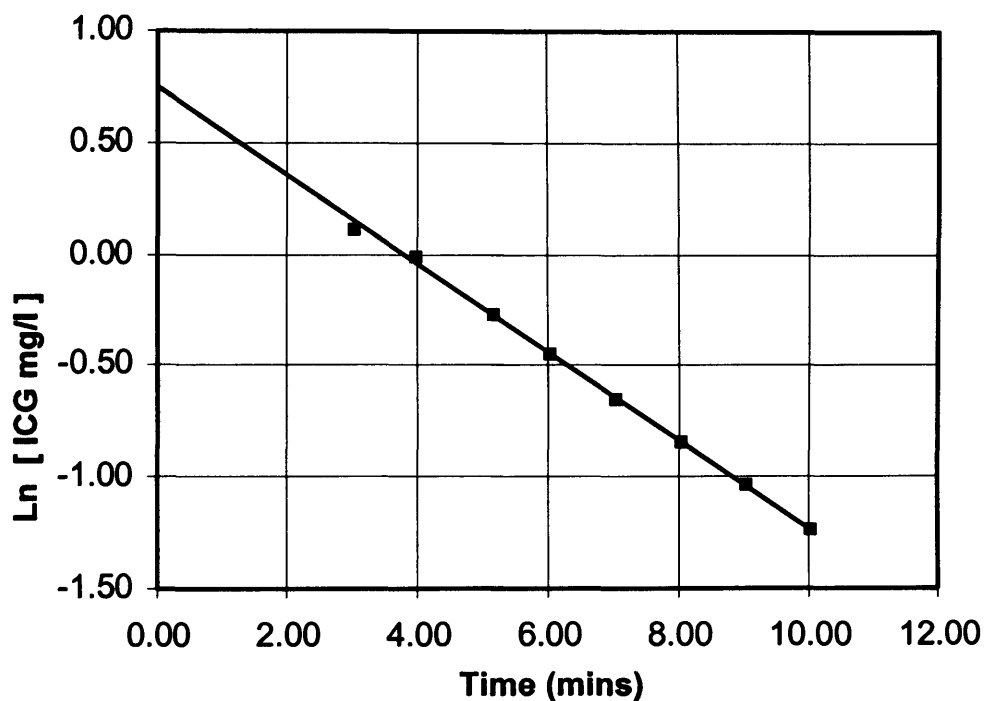


Figure 3 Comparison of 2 and 5 point calibration slopes
A = regression for 2 point calibration B = regression for 5 point calibration.

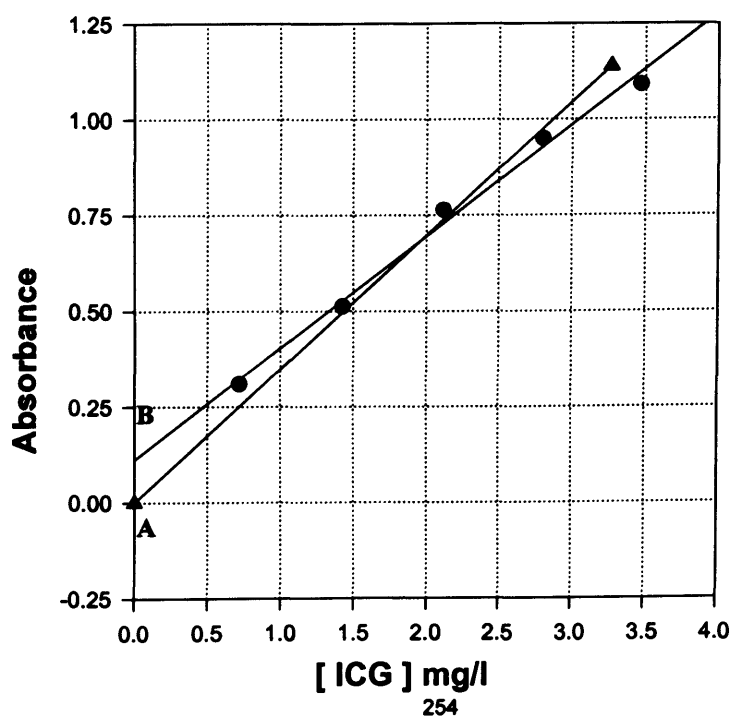


Figure 4 Bland Altman analysis of predialysis plasma volume measurements with during first (PV1) measurement at isovolaemic dialysis in nine patients. Reference lines indicate mean difference and 2 std. deviation.

PV1_{ISO_HD} = first absolute plasma volume measured during isovolemic dialysis PV_{PRE_HD} = predialysis supine absolute plasma volume using venous site fistula needle

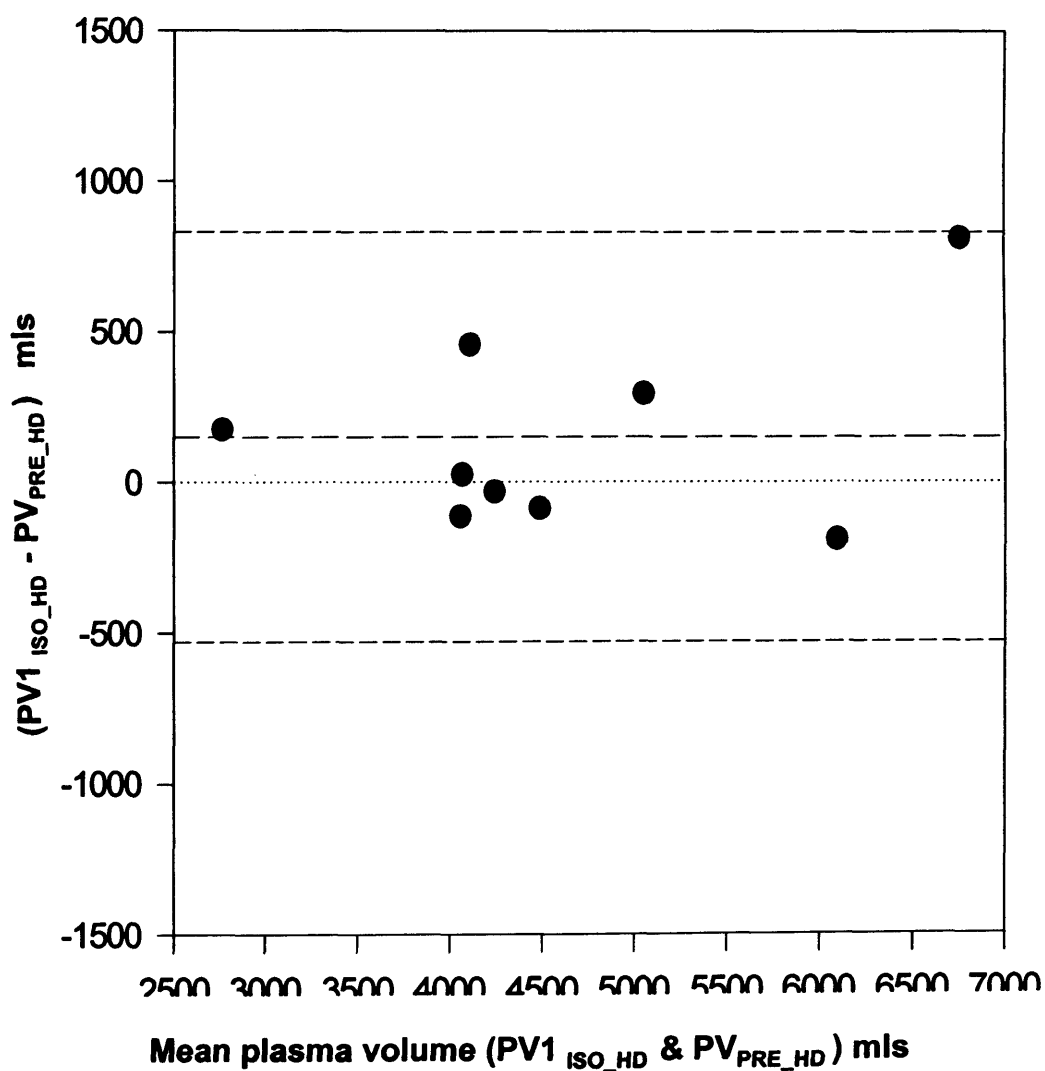
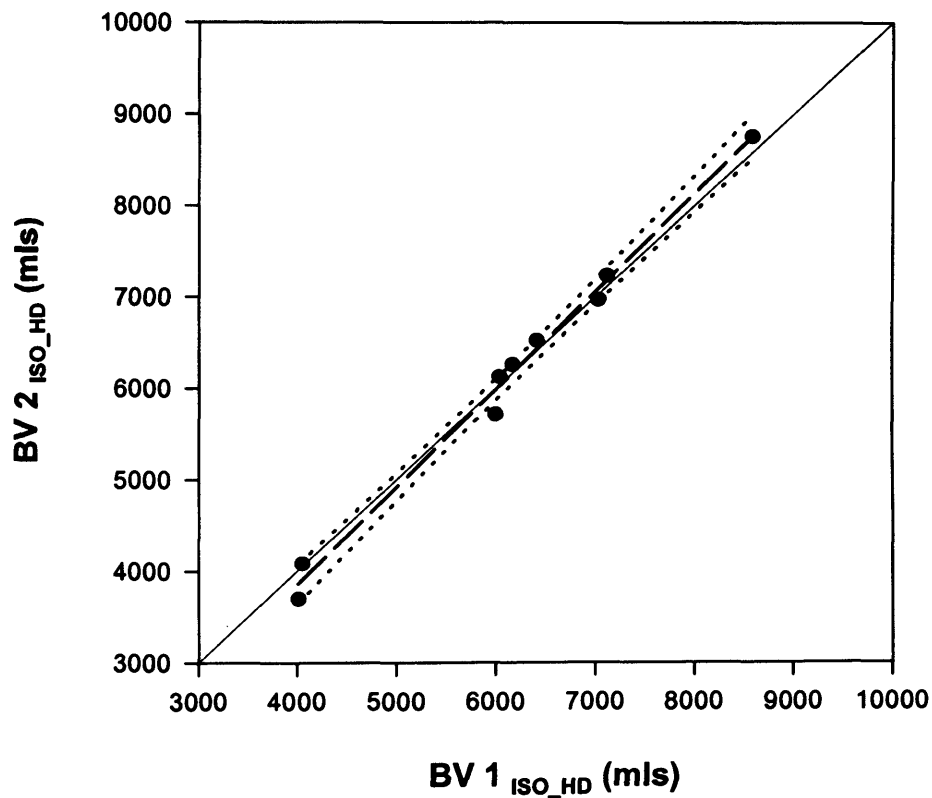


Figure 5 Regression line with 95% confidence intervals (slope) for consecutive blood volume measurements at 20 min intervals



BV 1_{ISO_HD} (first) versus BV 2_{ISO_HD} (second) in nine patients during isovolemic dialysis (----) line of identity. $R^2 = 0.99$

Figure 6 Regression line for plasma volume PV_{ICG} and extracellular fluid volume $ECF_{bioimpedance}$ with 95% confidence intervals (slope) during the initial isovolemic period on dialysis $R^2 = 0.73$

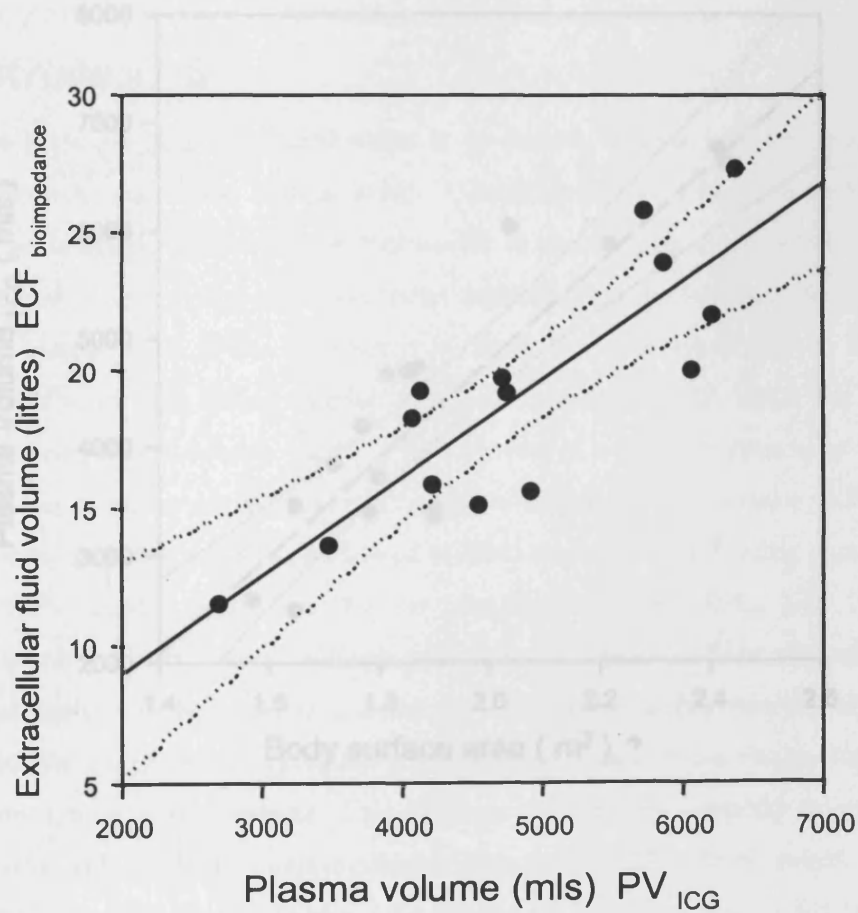
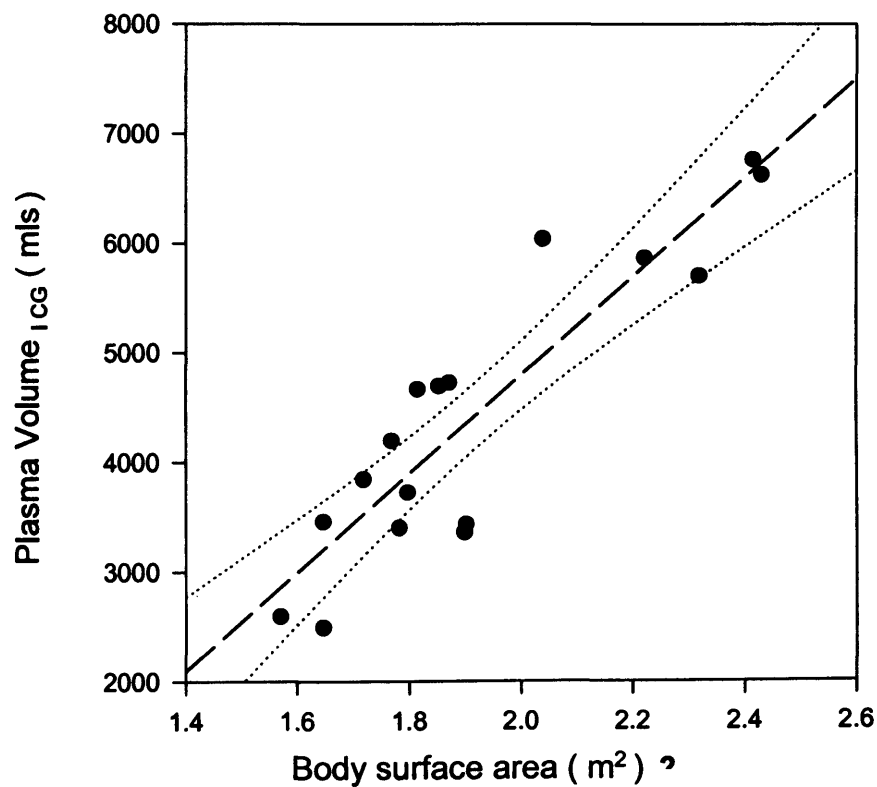


Figure 7. Relationship of plasma volume measured by indocyanine green and body surface area. The best fit linear regression is represented by $y = 4505.5x - 4217.7$ using Lev Marqdt least square procedure: $R^2 = 0.82$



Chapter 8

Tracking compartmental fluid shifts within the extracellular space during dialysis with profiled intermittent ultrafiltration

Introduction

The principle goal of ultrafiltration is to remove excess salt and water from an expanded extracellular compartment. Ultrafiltration (UF) induced depletion of the intravascular compartment however results in blood volume reduction. The changes in blood volume at any given time point depends upon the two opposing forces of UF and plasma refill (PR). Movement of fluid between interstitial to intravascular compartment (PR) during standard fixed rate continuous UF, is the key mechanism preserving blood volume. [259] PR however, is a difficult parameter to measure. The use of online monitoring of intradialytic relative blood volume (RBV) changes over the past few years have allowed indirect estimates of PR using parameters such as RBV changes normalised for the ultrafiltration rate (UFR). [260,105,131,134] However absolute plasma refill volumes during UF have not been studied.

Traditionally UFR is set at a constant rate for the entire haemodialysis session treatment based on the hypothesis that constant UF determines the gradual and linear reductions in blood volume. This assumes that the PR capacity in an individual patient remains fairly constant during a session of UF. RBV trends in different individuals tend to vary despite using the same UF prescription. [259] UF schedules have been tailored, modified or interrupted with the theoretical assumption that this may optimise movement of fluid between compartments (PR) to provide a more physiological regimen for the individual and reduce hypotension during haemodialysis. However clinical studies have failed to show benefit in reduction in hypotension episodes with various intermittent UF profiles. [261]

The purpose of this analysis was to examine directly measured plasma volume, blood volume changes and refill responses within compartments of the extracellular volume and their relationships with vascular stability during profiled intermittent ultrafiltration during haemodialysis.

Methods

Twelve stable chronic uremic patients on maintenance high flux HD were studied over 12 dialysis treatments. Each patient was studied during a routine dialysis session using a standard dialyser and urea kinetic model [112] and monitored using real time ultrasonic blood volume monitor. Four steps of UF (3L/hr) were applied which allowed intervening rest period and attainment of steady state blood volume. Ultrafiltrate volume was removed in a stepped manner (40%-20%-20%-20% of target

UF volume) to achieve the clinically prescribed dry weight unless hypotension ensued at which point the UF was stopped. Absolute plasma volume measurements were made using Cardiogreen at four steady states of relative blood volume (see chapter 7). Blood samples were obtained to determine the haematocrit and derive absolute blood volumes from the plasma volume and haematocrit. (see Chapter 7). Patients were monitored using BP (Oscillometric) and bioimpedance (multifrequency Whole Body Bioimpedance, Hydra, Xitron Technologies) to measure extracellular fluid volume (ECF). Area under RBV decay during UF was integrated to derive the refill divergence. (see Chapter 5). PR was calculated as the difference between the UF volume removed and plasma volume reduction between the measurement time points.

PR and change in interstitial fluid volume during UF were derived from the following equations :

$$J_{REF} (PRV) = J_{UF} - \Delta PV \quad \Delta IFV = \Delta ECF - \Delta ABV$$

Jref (PRV) = Plasma refill volume, Juf = ultrafiltrate removed

PV = plasma volume (ICG) IFV = Interstitial fluid volume (IFV)

ECF = Extracellular volume (Bioimpedance), ABV = absolute blood volume (ICG)

ECF was also derived post dialysis using Guyton formula [78] and compared with the measured bioimpedance values. The characteristics of these patients are described in table 1. All patients were ultrafiltered to their clinical dry weight.

Statistics

Statistical methods used were Bland Altman analysis for comparison of methods, t test for comparison of means ($p < 0.05$), linear regression and Pearson correlation tests. Statistical analysis was performed using SPSS software.(see methods)

Results

ECF changes during UF

The changes in the various compartments within the extracellular space between the start and end of the dialysis session are listed in Table 2. The mean UF volume removed (2406 ± 808 mls) correlated strongly with the mean ECF change (-2496 ± 758 mls; $r=0.92$) and intradialytic weight reduction (2.6 ± 0.79 kg; $r=0.96$) ($p < 0.001$). Predialysis extracellular volumes ECF (20.5 ± 3.6 litres) were expanded but lowered significantly at the end of treatment (ECF 18.4 ± 3.5 litres ($p < 0.01$)). The postdialytic measured ECF (bioimpedance) overestimated the predicted (Guyton) by 597 ± 406 mls ($r=0.93$ $p < 0.001$).

Changes in PV and ABV during UF

The mean absolute plasma and blood volume at the start of ultrafiltration were 60.4 ± 7.3 and 88.8 ± 10.6 mls/kg respectively. Mean percentage ABV reduction between the start and end treatment was -17.1 ± 8.6 %. Mean RBV reduction -9.34 ± 5.65 % with a wide variation between -18.4% to -0.5% . The net percentage circulatory plasma and blood volume change between the start and end of UF had a weak and nonsignificant relationship with UF volume removed.

Relationship between BP, plasma and blood volume

The measured circulating plasma volume PV (42 ± 8.5 ml/kg), absolute ABV (67 ± 13.4 ml/kg) and relative blood volumes RBV (90.2 ± 5.7 %) were significantly lower during hypotensive episodes (MAP < 70 mmHg ; $p < 0.05$; $n = 7$) than at higher mean BP (PV = 53 ± 8.6 ml/kg; ABV = 79 ± 11.9 ml/kg; RBV = $96.4 \pm 4.4\%$). (Fig 1)

Systolic BP reduction was best predicted by the reduction in plasma & blood volume, change in ECF and body weight. ($p < 0.01$)

Relationship between ECF V ABV

Markedly elevated predialysis PV/ECF ratio (0.24 ± 0.03) and ABV/ECF ratio (0.4 ± 0.05) fell significantly by the end of treatment (PV/ECF 0.2 ± 0.03 ; ABV/ECF ratio 0.34 ± 0.05) ($p < 0.01$ for both parameters). The relationship between blood volume and postdialytic body weight superimposes on what would be predicted by a Guyton prediction curve whilst at the start of dialysis the blood volume is disproportionately high (Fig 2). The change in plasma volume between the start and end of dialysis weakly correlated with the reduction in RBV ($r = 0.61$, $p = 0.04$) and change in ECF ($r = 0.58$, $p = 0.04$).

The Plasma refill quantification

The reduction in ECF correlated with plasma refill volume (PRV) ($r = -0.78$; $p < 0.01$). The mean reduction in interstitial volume - 1293 ± 751 ml (derived from difference between UFV removed and ECF change) was nearly identical to the mean calculated plasma refill volume PRV (1232 ± 715 ml $R^2 = 0.74$) (difference between change in absolute plasma volume and UFV). (Fig 3) The reduction in RBV directly correlated with PRV ($r = -0.83$, $p < 0.01$) Proximity to dry weight was associated with poorer refill capacity (PRV) ($r = 0.53$, $p < 0.05$). (Fig 4) The refill volumes for individual UF pulse were significantly lower for the final step UF (PRV₁ 7.5 ± 4.3 ; PRV₂ 4.1 ± 6.1 ; PRV₃ 2.54 ± 6.5 ml/kg/ml of ultrafiltrate ($p < 0.01$; 1 v 3). (Fig 5) PR during the first UF step did not correlate with predialysis extracellular volume. The total refill volume (PRV) during the whole UF session was predicted by change in RBV and ECF change.

The integral area under the UF decay curve derived for the RBV decay during the first UF step (refill divergence LD₁ 3053 ± 2103 %. secs) strongly correlated to the refill volume PRV₁ ($R = 0.81$; $P < 0.01$) (Fig 6)

Discussion

In healthy individuals plasma volume occupies approximately 20% of the extracellular compartment. [Introduction, Chapter 5] The relationship between blood volume and body weight, abnormally skewed at the start of dialysis, approaches the predicted normal distribution curve described for euvoletic individuals. Fig 2 This suggests that the distribution of excess fluid between compartments of the extracellular space can return to normal on approaching dry weight provided there is adequate ultrafiltration. This state of normohydration is perhaps short-lived and difficult to sustain, due to the intermittent nature of the treatment.

The blood volumes at low MAP were significantly lower than at preserved systemic blood pressure suggesting that a preserved circulating blood volume is crucial for haemodynamic stability. The study supports the concept that most of the plasma refill is derived from the interstitial space. Despite this the proportional reduction of ECF during high flux dialysis is contributed to by larger changes in circulating blood volume than previously thought. [262] The compensation is two-fold : from the interstitial compartment and intravascular refill.

We found a significant relationship between the plasma refill and haemodynamic stability. Schroeder et al on the other hand failed to demonstrate correlations of plasma refill rate with UFR, haematocrit change or heart rate using indirect estimates of refill with Critline monitoring during constant UF. [263] The presence of a high UF rate predisposes to larger proportional changes in the vascular compartment and haemodynamic instability.

In this study, refill volume corrected for body size and volume of the ultrafiltrate decreased during dialysis and was significantly lower in the last hour of dialysis within 0.5 kg of the post dialytic weight. Variation in refill volume was unpredictable except for

that during the last hour of dialysis. Schneditz [212] measured plasma density changes and, using a relationship based on Starling hypothesis and an open two-compartment model, derived a filtration coefficient of $5.6 \pm 1.4 \text{ ml}/(\text{min} \times \text{mmHg} \times 50\text{kglbm})$. Keshaviah [264] demonstrated that changes in plasma protein concentration accurately fit with plasma volume changes in a mathematical model, in which changes in plasma volume are assumed to be governed by plasma oncotic pressure changes. Ilimura et al [265] found that the refilling coefficient varies widely from case to case and decreases during haemodialysis. Refill may also be governed by vasoactive and other reflexes that have interindividual variation. The initial blood volume and the modifications in venous and arterial tone during HD can induce proportional modifications in hydrostatic capillary pressure, the extent of which is not uniformly transmitted to all tissues. [266] Hence the equilibrium of forces across the capillary wall and filtration coefficient can vary considerably.

The use of ultrafiltration pulses with intervening rest periods to help optimise target weight and allow for more haemodynamic stability has been questioned. The group suggested that a linear profile may be preferable since intermittent profiles may aggravate vascular instability. Although intermittent profiles may allow normalisation of the compartmental volumes and their distribution postdialysis, high UF rates predispose to intradialytic haemodynamic instability due to variable refill capacities. Moreover blood volume decline leading to hypotension may be multifactorial and may depend on peripheral resistance and reflex control of the circulation such as systemic compliance; action of feedback mechanisms working on venous unstressed volume; plasma oncotic pressure; and, especially, capillary wall permeability and interstitial space elastance. [267] A gradually decreasing UF profile with negligible UFR within the final 0.5 – 1kg of the total UF volume may serve as the best profile in many patients.

The current interpretation of relative blood volume changes assume that no fluid shifts occur during blood volume stability and that net refill is absent during a flat blood volume profile. Whether apparent blood volume recovery during interrupted UF is entirely due to refilling fluid is not proven.

These results emphasise that the volume shift in the ECF and plasma refill play a significant role in maintaining haemodynamic stability. Hypotension may be dependent on the maintenance of a critical absolute blood volume which is patient specific. The blood volume changes and variable and unpredictable refill rates may explain the high incidence of intradialytic complications observed with standard dialysis despite technical advances in ultrafiltration.

Knowledge of PRV and compartment shifts helps in understanding intradialytic events and may help to improve techniques of fluid removal. Individualised ultrafiltration profiles that can adapt to refill capacity of the patient may be more physiological. As both the UFR and PR rate variables can change during a single dialysis session, this supports the use of devices with automated continuous adjustments of the UFR and more refined profiling strategies.

Table 1 Patient characteristics

	Mean (SD)
Age (yrs)	59.2 (8.78)
Sex (m:f)	10 : 2
BSA (m ²)	1.99 (0.22)
Predialysis wt	84.3 (14.8)
Predialysis ECF	20.5 (3.6)
Postdialytic weight (kg)	81.7(14.5)
UF volume (mls)	2406 (808)

SD = standard deviation ; ECF = extracellular volume
 UF = ultrafiltrate volume; BSA = body surface area

Table 2 Compartmental Fluid shifts during UF

	Mean (±SD) (mls)
Δ ECF (mls)	-2496 (758)
Δ ABV (mls)	-1202 (626)
Δ PV (mls)	-1174 (444)
RBV %	-9.34 (5.65)
Δ IFV (mls)	-1293 (751)

RBV = relative blood volume (BVM)
 PV = plasma volume (ICG) IFV = Interstitial fluid volume (IFV)
 ECF = Extracellular fluid volume (Bioimpedance)

Figure 1 Boxplot showing significantly lower plasma, absolute and relative blood volumes during hypotension MAP < 70mmHg (n = 7) compared to nonhypotension (n = 30) ** = p < 0.01 * p < 0.05

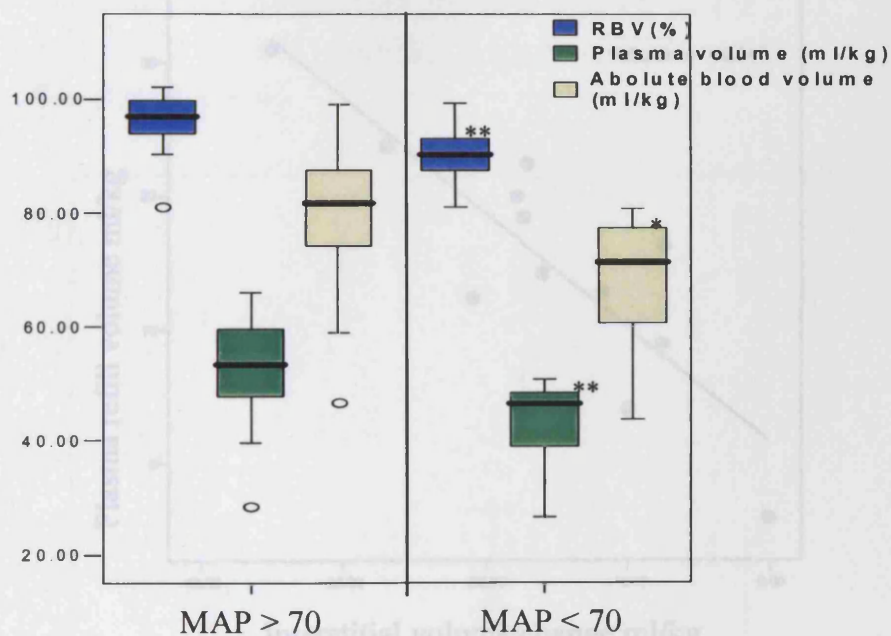


Figure 2 showing that disproportionately high blood volume at the start of dialysis (ABV 1) tend to assume a distribution post ultrafiltration (ABV 4) that would be predicted (Guyton) weight based formulae (Guyton_postABV)

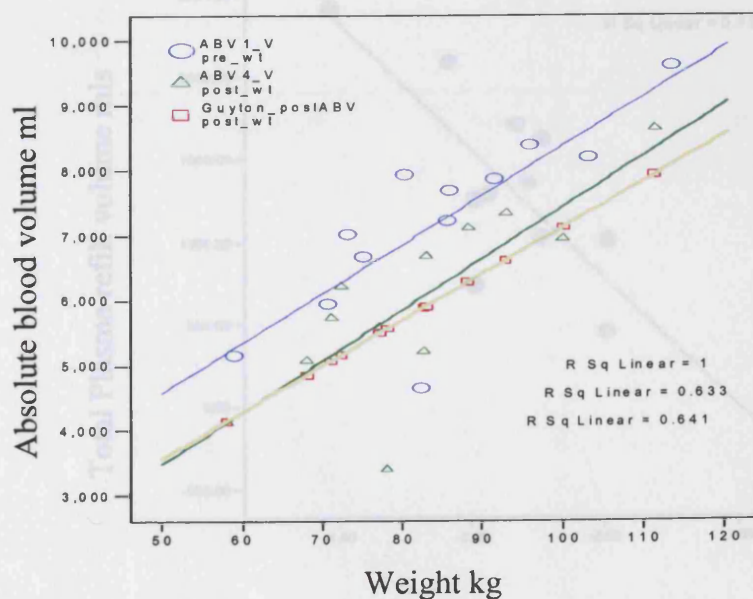


Figure 3 Linear regression between reduction in interstitial fluid volume and Plasma refill volume

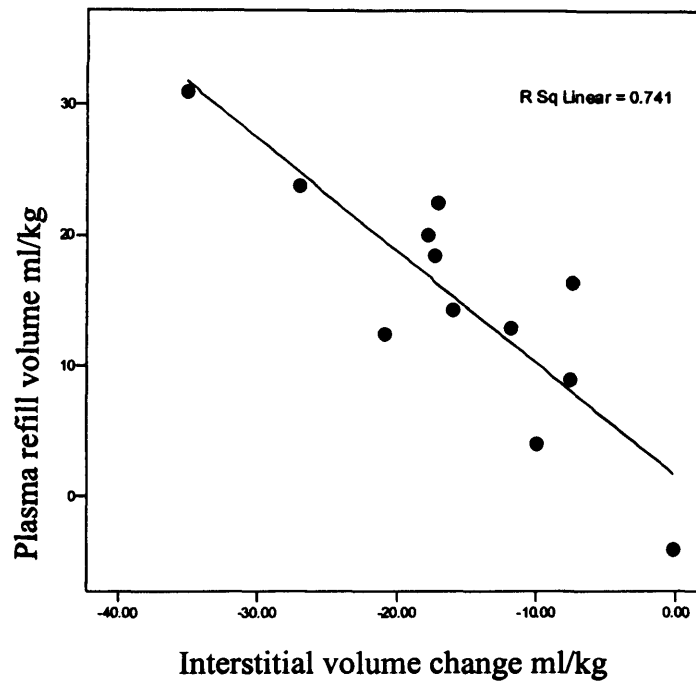


Figure 4 Linear regression between distance from target weight and Plasma refill volume

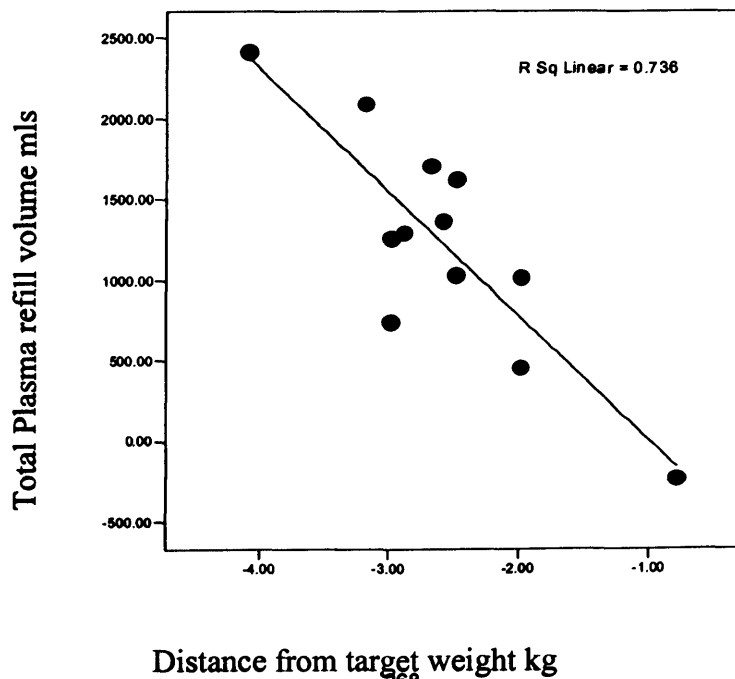


Figure 5 Plasma Refill volume ml/kg/ml ultrafiltrate for each step UF pulse Ref1 for first (UF1), Ref 2 mid (UF2 & UF3) and Ref 3 for final UF4 pulse
 *p<0.01 (1 v 4)

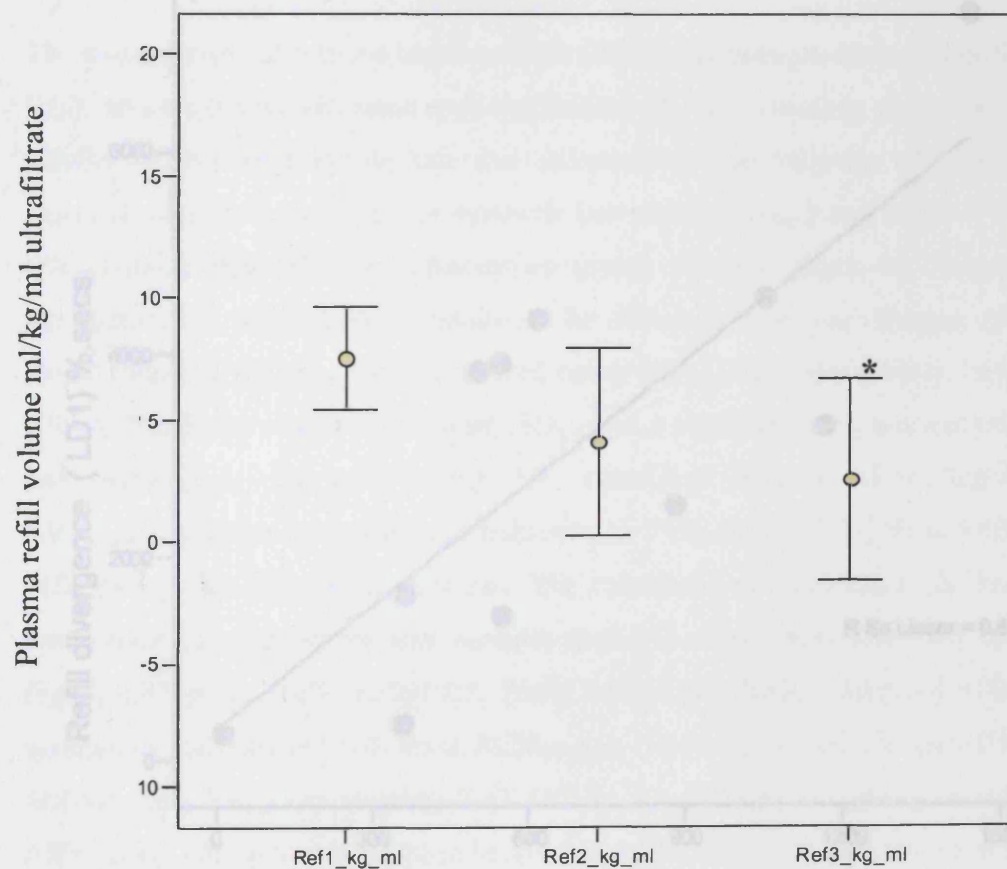
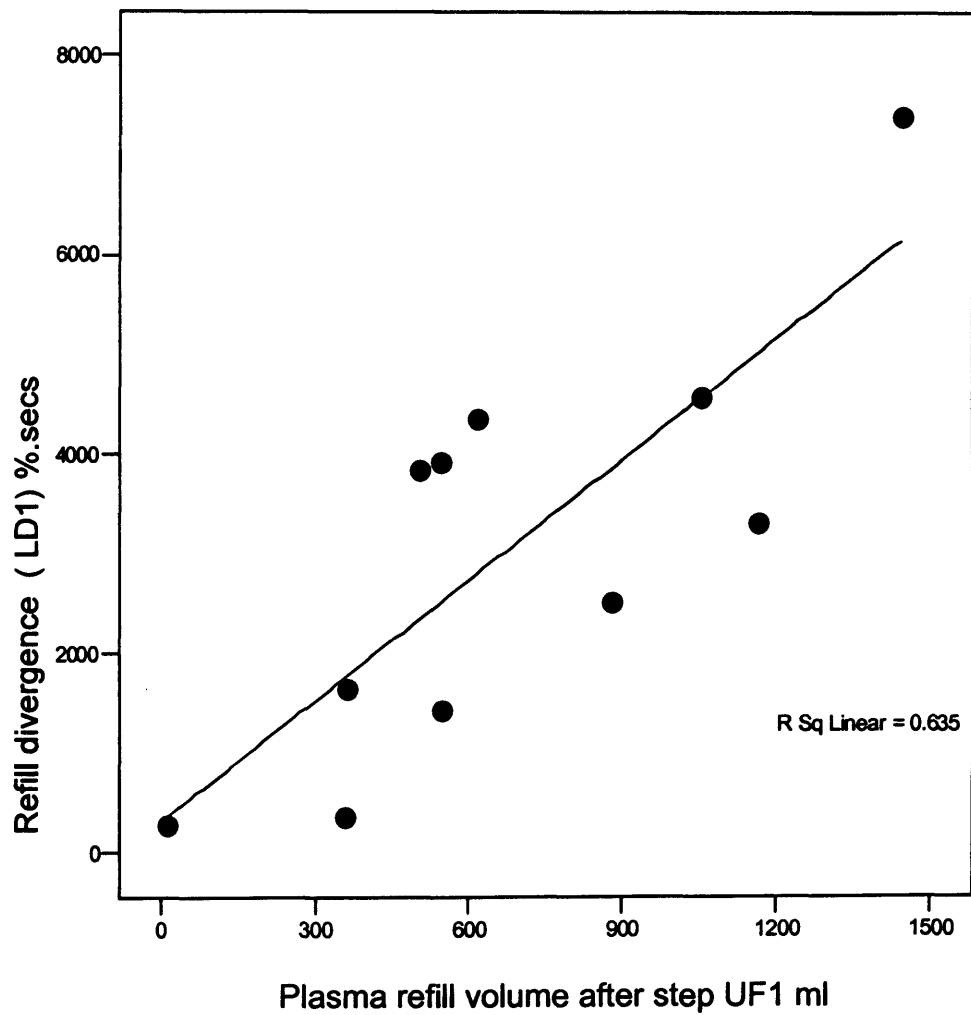


Figure 6 Refill divergence during the first step UF showed significant correlation with the plasma refill volume (PRV)



Chapter 9

The Fcell Ratio Increases During Ultrafiltration in Haemodialysis

Summary

The measurement of relative blood volume (RBV_{BVM}) changes during ultrafiltration (UF), assume a constant mass and distribution of its circulating components. The validity of the latter assumption was examined in ten subjects who underwent repeated direct measurements of systemic hematocrit (Hct_{sys}) and plasma volumes (PV_{icg}) using dye dilution (indocyanine green) at four stages of dialysis with intermittent UF. Subjects were monitored for ultrasonic RBV_{BVM} changes. Absolute blood volume ($ABV_{Fconstant}$) was derived using (PV_{icg}) and whole body hematocrit (Hct_w). The latter was obtained from Hct_{sys} and a constant F_{cell} assumption. PV_{icg} and $ABV_{Fconstant}$ changes during UF correlated closely ($R=0.98;p<0.001$). $ABV_{Fconstant}$ changes overestimated reduction in PV_{icg} during UF [Mean Difference MD -140 ± 202 ml] (Bland Altman). The calculated red cell mass ($RCM_{Fconstant}$) using $ABV_{Fconstant}$ however was variable ($p<0.01$). F_{cell} ratio was then corrected ($F_{cell1} 0.87\pm0.02$, $F_{cell2} 0.89\pm0.03$, $F_{cell3} 0.94\pm0.06$, $F_{cell4} 0.94\pm0.04$ $p<0.01$) to maintain a constant red cell mass $RCM_{Fvariable}$ (2146 ± 460 ml). When ABV was derived using PV_{icg} and variable F_{cell} ($ABV_{Fvariable}$) the MD of PV_{icg} changes and $ABV_{Fvariable}$ changes was negligible (-0.2 ± 35 ml). During intermittent UF, $RBV_{(BVM)}$ changes systematically underestimated percentage $ABV_{Fconstant}$ reduction [MD 7.7 ± 10.6 %]. When corrected for variable F_{cell} ratio $ABV_{Fvariable}$ and $RBV_{c(BVM)}$ differences were negligible [MD 1.2 ± 2.6 %]. It is postulated that varying F_{cell} ratio reflects a microvascular volume change with net fluid shift from the microcirculation to macrocirculation (intravascular refill). This can result in underestimation of the Hct_{sys} and RBV_{BVM} changes less than that predicted by directly measured changes in plasma volume.

Introduction

Technological advances have allowed the development of devices that can continuously and non-invasively monitor biologic constituents (haematocrit [Hct] and plasma protein concentration) during hemodialysis (HD) treatment. Hct and relative blood density changes online during HD have been advocated as tools for assessing blood volume (BV) changes induced by ultrafiltration (UF). [127] The assumption that changes in the measured systemic Hct (Hct_{sys}) result solely from circulating plasma volume (PV) changes induced by UF and that there is uniform mixing of a constant circulating mass of red cells and plasma components in the whole circulation during UF, form the basis of such indirect measurements.

Attempts to precisely quantify volume shifts in the vascular compartment using relative changes have been difficult as they often underestimate directly measured BV changes. [226] Apparent relative blood volume (RBV) changes therefore cannot be explained by PV depletion alone. Observational studies to analyse the RBV traces or determine the critical Hct for hypotension suggest wide degrees of inter-patient and intra-patient variability during haemodialysis with UF. [268] These imply that other physiologic mechanisms and alterations affecting distribution of the circulatory components during UF may influence these indirect estimates. [226]

Accurate measurements of red cell volume by radioactive isotopes show that the relative volumes of red cells and plasma in the circulation as a whole (whole body haematocrit [Hct_w]) differ from those found in the venous blood (Hct_{sys}). The difference between the systemic hematocrit in the macrocirculation [Hct_{sys}] and whole body hematocrit [Hct_w] is expressed as the Fcell ratio (Hct_w / Hct_{sys}). In the steady state this is due to a dynamic reduction in microvascular Hct during blood flow through the capillaries and venules (< 200 μm). This is also known as the Fahraeus effect [269] and depends on the capacity of the microcirculation. Reduction is greater in smaller vessels due to anomalous flow properties of blood. [270] The use of changes in Hct_{sys} to accurately reflect BV changes depends on the constancy of the relationship of Hct_w to Hct_{sys} during UF. The assumption of constancy of F cell ratio during dialysis with UF has not been investigated. This study examines the validity

of this assumption and hypothesises that there are significant changes in the microcirculation during UF that affect Hct redistribution and RBV changes.

Methods

Subjects

10 subjects (8 male) were studied using repeated measurements of PV and Hct during a single supine HD session with intermittent UF (3litres/hr). Four intermittent UF pulses were employed removing successively 40-20-20-20% of total UF volume between intervening rest periods. Subjects had received chronic haemodialysis for at least 6 months and had a stable dry weight. The presence of iodine allergy, eosinophilia, abnormal liver function tests, raised serum IgE levels or significant access recirculation within one month prior to the study were exclusion criteria. The North Herts Ethical Review committee approved the study. All patients gave informed consent. All were treated by thrice-weekly high-flux bicarbonate HD (Fresenius 4008E) using polyamide membranes and AV fistulae. Blood flow rates were in the range of 350-450 ml/min and the mean sessional Kt/V was 1.24 +/- 0.16. [112]

Plasma volume measurement

PV was measured by dye dilution using indocyanine green. [152] Four estimates of PV ($i = 1 - 4$) were obtained during a single haemodialysis session in each patient. All measurements were made in stable supine position. UF was commenced after an equilibration period of twenty minutes from the connection to the extracorporeal circuit. PV measurements were made before the start and at the end of the first and fourth UF pulse (Fig 1) when steady state BV conditions were obtained on the RBV monitor (RBV plateau with variations of less than 0.1% over at least ten minute period).

Tracer

The tracer used was Cardiogreen (ICG Green TM Sterile indocyanine green USP Fluka), a tricarboyanine dye, mol wt 775, with an absorption peak at 805nm. The dye is non-toxic, confined to plasma, not subject to extra-vascular distribution, and

not metabolised or degraded. After equilibration, the dye decays fast in an exponential manner. It is exclusively taken up by the liver and has a plasma half-life 2-3 min. [153]

Procedure for determination of plasma volume

Prior to each dye injection, blood was withdrawn for Hct and baseline plasma blank in heparinised syringes. ICG (25 mg) was dissolved in 10 ml of sterile aqueous solvent to produce ICG solution of 2.5mg/ml. The dye (10mg) was then injected as rapidly as possible into a venous port beyond the bubble trap. All syringes were weighed on a precision scale before and after the injection to determine the precise quantity injected. Exactly 3 minutes after the end of the injection, sampling was commenced from the arterial port in heparinised syringes at 1-min intervals for 10 min (8 samples). Samples were centrifuged at 3000 rpm for 10 minutes. The plasma blank sample was used to determine the baseline background absorbtion at 805-nm wavelength. The absorbtion of ICG dye in the timed plasma samples were then compared against the baseline at the same wavelength. [153] A 5-point calibration was performed for each BV measurement using the blank plasma sample just before dye injection. (see Chapter 7)

Analysis

The natural logarithms of the measured ICG dye concentrations were plotted against time for each PV measurement and the best fit linear regression obtained. The logarithm of the dye concentration at $t = 0$ was obtained by extrapolation. The antilog of this yielded the initial dye concentration in plasma at the time of injection. Plasma volume (PV_{icg}) was calculated according to the equation:

$$Plasma\ volume^{[247]} = \frac{dye\ infused\ (mg)}{plasma\ dye\ concentration\ (mg / l)} \quad \text{Equation 1}$$

Haematocrit determination

Hct_{sys} was measured using aperture impedance counters (Coulter-STKS). [271] All patients were monitored with relative blood volume (RBV_{BVM}) monitor [127] and oscillometric blood pressure.

Procedure for determination of absolute blood volume and red cell mass

1. Absolute blood volume ($ABV_{Fconstant}$) [247] estimates were derived for each PV estimate and corresponding measured Hct_{sys} adjusted by a factor of 0.86 to correct for the difference between the Hct_{sys} and Hct_w (constant F cell ratio)

$$ABV_{Fconstant} = \frac{\text{plasma volume}}{1 - (Hct_{sys}) * Fcell^{[269]}} \quad \text{Equation 2}$$

2. Total red cell mass ($RCM_{Fconstant}$) estimates were then derived from each $ABV_{Fconstant}$ estimate and the corresponding Hct_w using the relationship:

$$RCM_{Fconstant} = ABV_{Fconstant} \times Hct_w \quad \text{Equation 3}$$

3. Subsequently separate F cell ratios ($Fcell_i$) were calculated for each of the four plasma volume (PV_i) and corresponding Hct_{sys} [$(Hct_{sys})_i$] values obtained during each dialysis session. The calculation assumed that a) RCM remained constant throughout the dialysis session and b) that absolute blood and plasma volume changes during UF were effectively identical.

4. $(ABV_{Fvariable})_i$ was then recalculated from equation 2 utilising the appropriate PV_i and $(Hct_{sys})_i$ measurements and the corresponding $(Fcell)_i$

5. The differences in F cell ratio between the first [$(Fcell)_1$] and subsequent [$(Fcell)_i$] measurements, were used to correct the RBV reading (corrected reading designated as RBV_c) obtained from the RBV monitor at the second (RBV_2), third (RBV_3) and fourth (RBV_4) measurements:

$$RBV_{ci} = RBV_i \times \{1 - [(Fcell)_i - (Fcell)_1]\} \quad \text{Equation 4}$$

Where $i = 2 \text{ to } 4$

Statistics

Statistical methods used were Bland Altman [246] analysis required for comparison of methods, Student's test for comparison of means ($p < 0.05$), linear regression and Pearson correlation tests. Statistical analysis was performed using software package Sigmaplot (version 2.01).

Results

The subjects had a mean age of 61.6 ± 4.8 yr, a mean dry body weight of 83 ± 17 kg and a body surface area of 2 ± 0.2 m². The mean UF volume removed was 2298 ± 845 ml and there was a net reduction in PV during dialysis of 1218 ± 474 ml. The mean reduction in weight during dialysis was 2.48 ± 0.9 kg.

The derived values for $ABV_{Fconstant}$ from directly measured plasma volumes (PV_{icg}) and hematocrit (*Equation 1*) are shown in Table 1. PV changes and BV changes were highly correlated ($R = 0.98$; $p < 0.001$). When the mean red cell mass ($RCM_{Fconstant}$) was calculated using ($ABV_{Fconstant}$) and the measured hematocrit, there were significant differences between the calculated values obtained at each measured PV_i ($p < 0.01$ Fig 2a).

The assumption of a constant circulating RBC mass during UF is violated unless there is a progressive increase in F cell ratio. Hence the F cell ratios were corrected ($Fcell_1$ 0.87 ± 0.02 , $Fcell_2$ 0.89 ± 0.03 , $Fcell_3$ 0.94 ± 0.06 , $Fcell_4$ 0.94 ± 0.04 ; $P < 0.01$ $Fcell_1$ v $Fcell_3$, $P < 0.001$ $Fcell_1$ v $Fcell_4$) assuming a constant circulating red cell mass ($RCM_{Fvariable}$ mean 2146 ± 460 mls $p = ns$: Fig 2b). The resulting ABV estimations ($ABV_{Fvariable}$) are depicted in Table 2. The corrected mean absolute blood volume obtained at the end of ultrafiltration was 72.2 mls/kg (Table 2 ABV 4).

Absolute blood volume changes ($ABV_{Fconstant}$) systematically overestimated plasma volume changes (PV_{icg}) with a mean difference of -140 ± 202 mls (Bland Altman analysis: Fig 3a). When corrected for F cell variation, the mean difference of plasma and BV changes were negligible (-0.2 ± 35.8 ml: Fig 3b). The change in F cell ratio during UF (Fig 4) correlated with the UF volume removed (R 0.32; $P < 0.05$).

Relative BV changes (%) significantly underestimated the percentage reduction of absolute BV ($ABV_{Fconstant}$) between the four measurements (Table 1) with a mean difference (\pm std deviation) of 7.7 ± 10.6 % (Fig 5a). When corrected for varying F cell ratio, the mean difference between change in corrected RBV (RBV_C Equation 4) and absolute BV ($ABV_{Fvariable}$ Table 2) was only 1.2 ± 2.6 % (Fig 5b).

Discussion

The assessment of volume shift using systemic haematocrit or plasma density is based on mass conservation. The first assumption of a constant total circulating red cell mass during a dialysis session is true in the absence of haemolysis or blood leak. This parameter is only likely to fluctuate over time with changes in erythropoietin treatment. Red cell volume may vary with plasma osmolality, but the degree of change is small even across extreme variations in dialysis fluid sodium concentration [272]. A second assumption is of constant homogenous distribution of these components between the macro and the microcirculation defined by F cell ratio [269] throughout dialysis. A constant F cell ratio implies that there is no net microvascular volume change. This study demonstrates that both these assumptions cannot be true at the same time. The assumption of a constant circulating RBC mass during UF is violated unless there is a progressive increase in F cell ratio during UF.

A model of circulatory changes during UF with or without microvascular changes, can be hypothesised based on these results (Fig 6). In the steady state (Fig 6a), the intravascular volume (V_b) can be divided into two compartments with proportional distribution of plasma and red cells: the macrocirculation ($V_{mac} = 60\%$: $H_{sys} = 0.35$) and the microcirculation ($V_{mic} = 40\%$: $H_{mic} = 0.233$). If UF were associated with no change in F cell ratio, a proportional volume change would occur in each compartment, and the observed rise in Hct_{sys} would purely reflect the volume removed from the macrocirculation (Figure 6b). However the observed underestimation of rise in Hct_{sys} suggests that there are additional physiological factors in operation. There are two main possibilities. There could be loss of red cell mass from the systemic circulation, which is unlikely, or there could be intravascular refill from the micro- to macrocirculation. The latter mechanism results in a new

steady state of the microcirculation and a higher F cell ratio (Figure 6c). It is strongly supported by the almost complete correction of systemic RBV underestimation by use of a varying F cell ratio.

The ratio of the red cell mass to PV differs in the venous system, the capillaries, the splenic blood pool and in different organ beds. Any perturbation provokes a proportional change in microvascular and systemic haematocrit, which can be represented by the constant α , the value of which is approximately 0.66 based on studies of different microvascular beds [273,248]. Each microvessel generation could constrict in a heterogenous manner. These heterogeneties can be averaged by using the value α . Since α is fairly constant, any rise in F cell ratio entails a reduction in V_{mic}/V_b (see Appendix 7). In the hypothetical example depicted in Figure 6, these relationships predict a reduction in V_{mic}/V_b from 21% (Fig 6b) to 12% (Fig 6c). Such models have been used to describe the microvascular circulation under different pathophysiologic conditions. [274]

Observations on the hepatic and pulmonary circulation indicate that changes in microvascular volume lead to transient changes in the Hct or density of blood flowing from these organs. [275] If the circulation is subjected to any perturbation, which changes the microvascular volume, this contributes to part of the measured change in the Hct_{sys} . There is no evidence to suggest significant alterations in capillary permeability characteristics during UF. [274, 212] Morphometric data indicates that 40 – 50 % of BV resides in the microcirculation. Direct microvascular measurements suggest that during haemorrhage, <200 μm diameter venules form the major reserve capacity of the circulation. Large volumes may be shifted from the micro to the macrocirculation, reducing the effect of BV loss.

These findings suggest that microvascular change induced by UF is an important factor influencing the Hct_{sys} . This seems to be accentuated at later stages of UF, when a rise in Hct_{sys} may not occur, despite hypovolemia. This is likely to be due to intravascular refill. Studies using tagged RBC have suggested that during hypovolemia due to UF, mobilisation of blood from the splanchnic region occurs as a compensatory mechanism [276].

This study provides evidence of dissociation between indirect RBV measurements and direct BV changes measured by indocyanine green during haemodialysis, most apparent at later stages of dialysis with ultrafiltration.

The absolute blood volume estimates with ICG in this study corrected for F cell variation (mean 72 ml/kg at end of UF) approximate physiological values in the nondialysis population [277]. Lower blood volume estimates obtained using radioisotope methods in subjects prior to the start of dialysis in a previous study [242], are perhaps due to smaller body size, methodological variations and differences in hydration status of the subjects studied.

The mass of protein in the vascular space at the time of the dye measurement is irrelevant for the plasma and blood volume determination. The dye simply binds instantaneously to the available circulating protein mass. This study assumes that during blood volume steady state with no UF and a relative small sampling period there is dynamic equilibrium and negligible net flux of protein across capillary membrane. Relative blood volume measurements are also based on the same assumption allowing comparison between two methods. Changing vascular refill rates despite apparent steady state conditions in the relative BV profile seem unlikely to account for this, given the degree of underestimation. Although the ultrasonic RBV monitor has a very low noise signal ratio, momentary fluctuations may introduce potential errors.

However, the dissociation is almost eliminated when the variation in F cell ratio is considered, suggesting that intravascular refill and regional blood flow redistribution during UF significantly affect RBV measurements. These observations and others, such as the RBV changes observed during maximal exercise on dialysis [278], support the notion that RBV measurements can be significantly affected by procedures that induce changes in the recruitment of the microcirculation.

Conclusion

Microcirculatory changes lead to volume shifts from the micro to the macrocirculation with adjustment of the macrovascular Hct_{sys} during UF. A compliant microcirculation acts as a blood reservoir allowing volume compensation during UF. Such redistribution lead to a progressive rise in the F cell ratio during UF in the presence of a constant red cell mass. Hence the assumption of a constant and

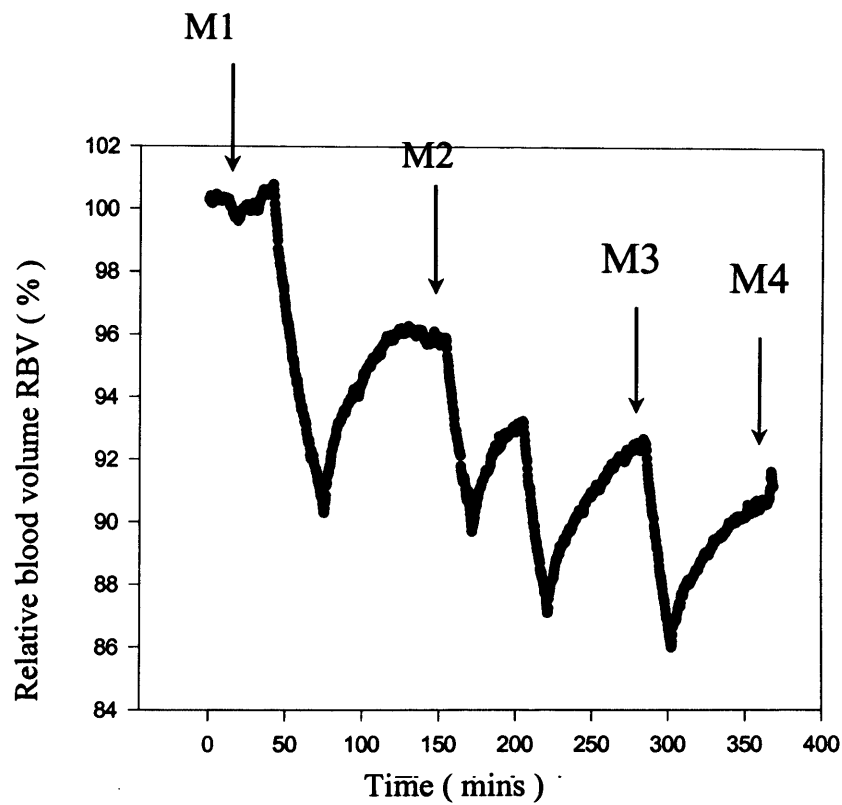
homogenous Hct distribution during UF is invalid, and the use of Hct_{sys} change as the sole determinant of PV change could be erroneous. Both the density and the Hct equations employed to determine changes in RBV, ignore volume redistribution between the macro and microcirculation. This study can serve as the basis to design experiments to characterise the mechanisms producing microvascular change during UF.

Subjects	ABV1	ABV2	ABV3	ABV4
1	8033	7139	6157	5944
2	5195	4625	3598	3977
3	5994	5578	5262	4881
4	4682	4670	3507	3269
5	8248	7450	6905	6717
6	7726	6849	5790	5093
7	7081	6854	5960	5504
8	7283	6791	6633	6392
9	9670	9450	8781	8363
10	6717	6387	6059	6018

Table 1 Absolute blood volumes ($ABV_{F_{constant}}$) derived by using measured plasma volume, hematocrit and constant F cell ratio (0.86) in ten subjects during hemodialysis

Subjects	ABV1	ABV2	ABV3	ABV4
1	7995	7139	6348	6157
2	5165	4625	3728	4125
3	5964	5578	5455	5074
4	4660	4670	3648	3405
5	8208	7450	7119	6945
6	7701	6849	5928	5220
7	7040	6854	6196	5734
8	7242	6791	6903	6679
9	9621	9450	9062	8642
10	6684	6387	6248	6215

Table 2 Absolute blood volumes ($ABV_{F_{variable}}$) derived by using measured plasma volume, hematocrit and variable F cell ratio in same ten subjects during hemodialysis



Blood volume changes during Stepwise Ultrafiltration M1-M4 plasma volume (ICG) measurements

Figure 1 Relative blood volume profile during intermittent ultrafiltration in a subject on hemodialysis. Arrows indicate the timing of the four steady state hematocrit and direct plasma volume (ICG) measurements. M1 Measurement before onset of ultrafiltration (UF); M2 after 40% UF; M3 after 80% UF; M4 after 100% UF removed.

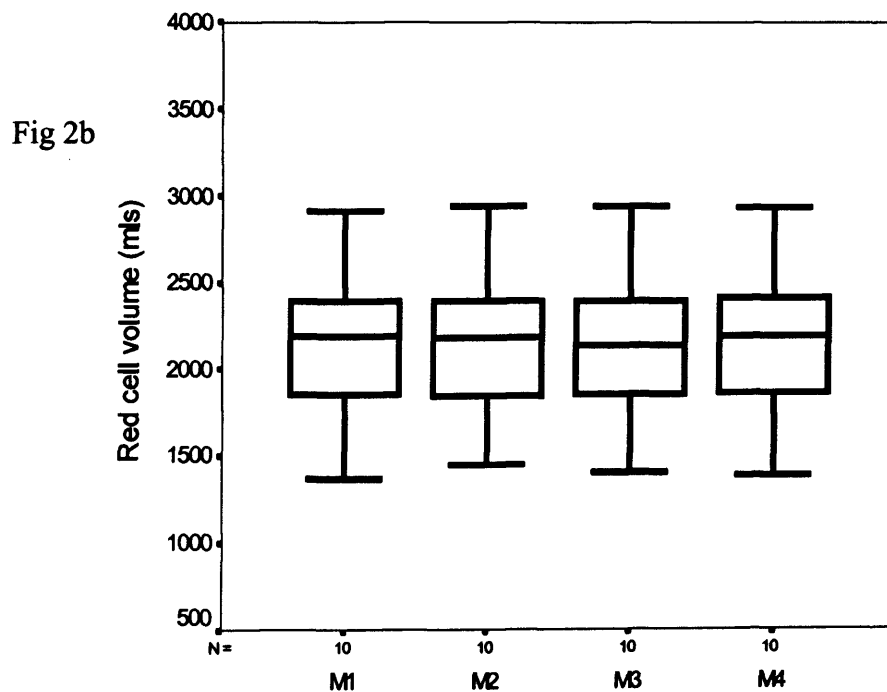
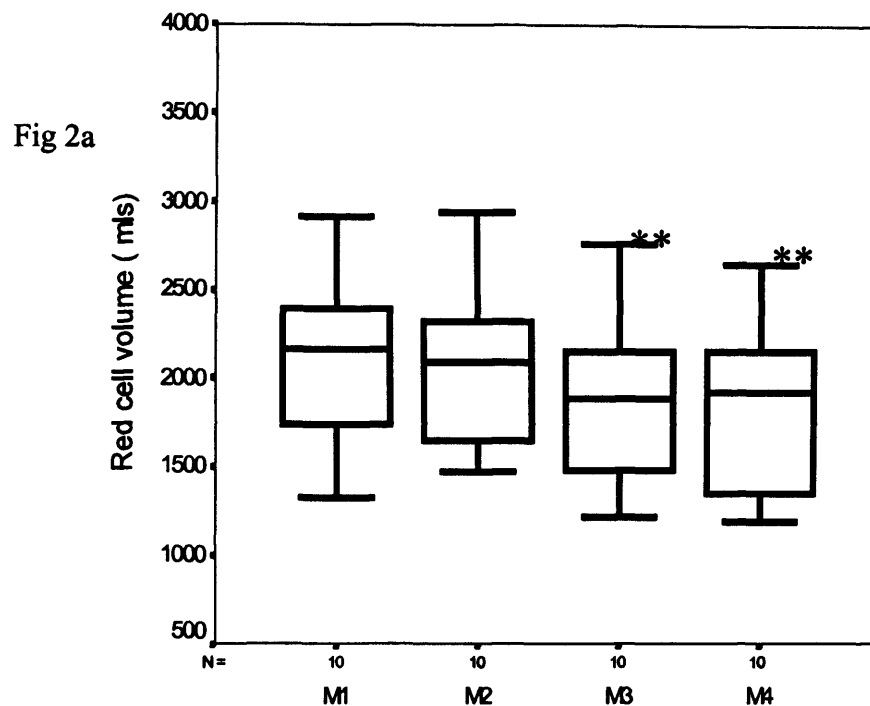


Figure 2 (a) Box plot showing inconstant total red cell volume ($RCM_{F_{constant}}$) in 10 subjects during dialysis using constant F_{cell} (** $P < 0.01$) (b) Box plot showing constant red cell volume using variable F_{cell} ratio ($RCM_{F_{variable}}$; NS).

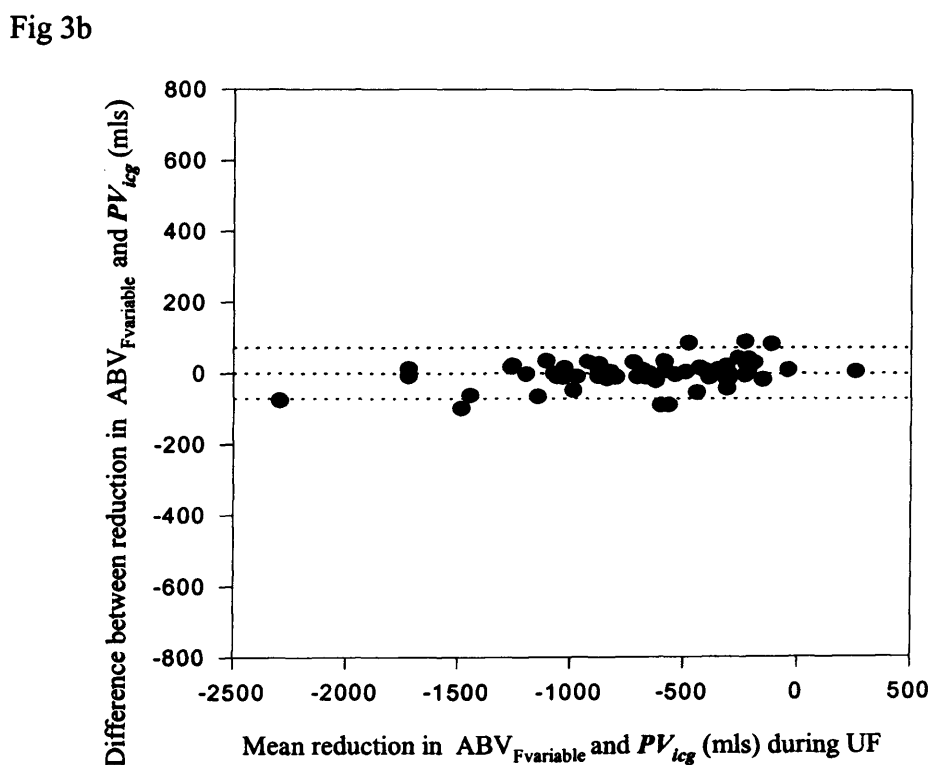
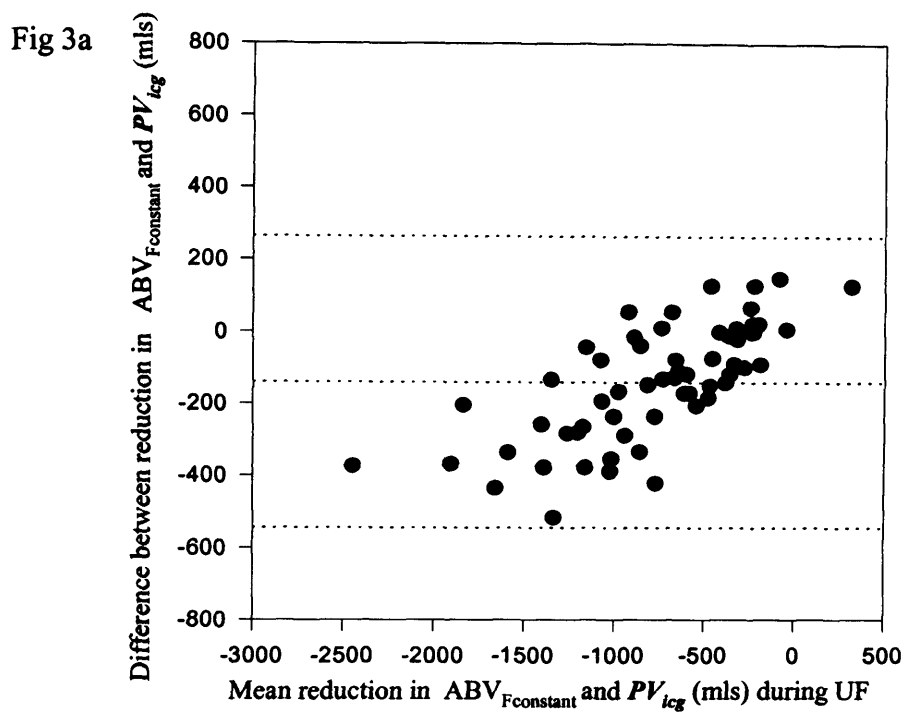
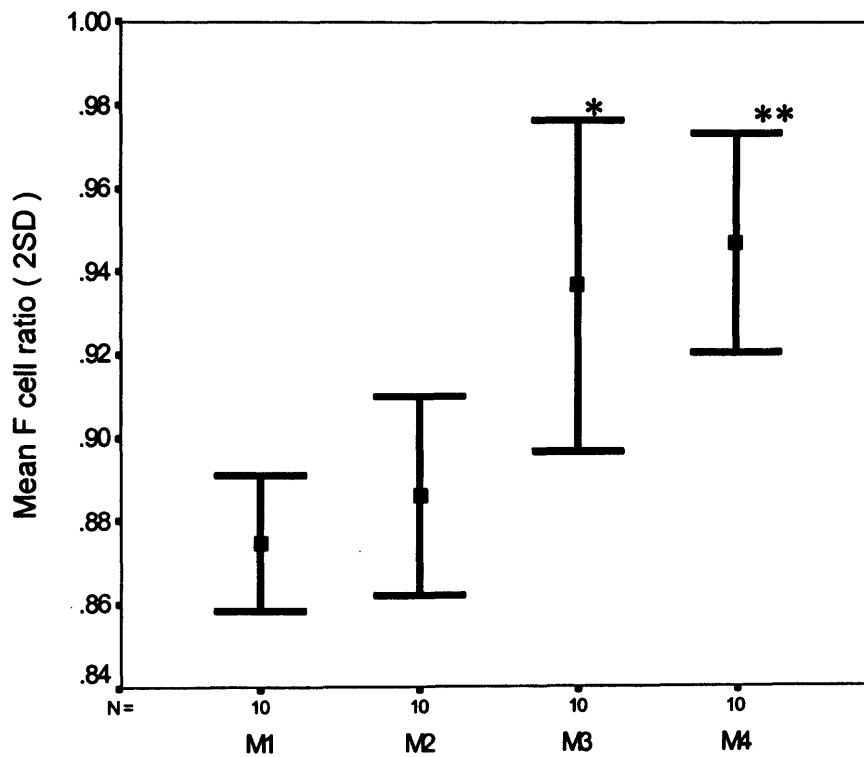


Figure 3 Bland Altman analysis comparing directly measured plasma volume changes and absolute blood volume changes derived using constant F cell ratio ($ABV_{Fconstant}$) mean difference -140 ± 202 mls (3a) and variable F cell ratio ($ABV_{Fvariable}$) negligible mean difference -0.2 ± 35.8 mls. (3b) Reference lines indicate mean difference ± 2 SD

Figure 4 Progressive rise in F cell ratio obtained in ten subjects at four steps of intermittent ultrafiltration (*p<0.05, **p<0.01)



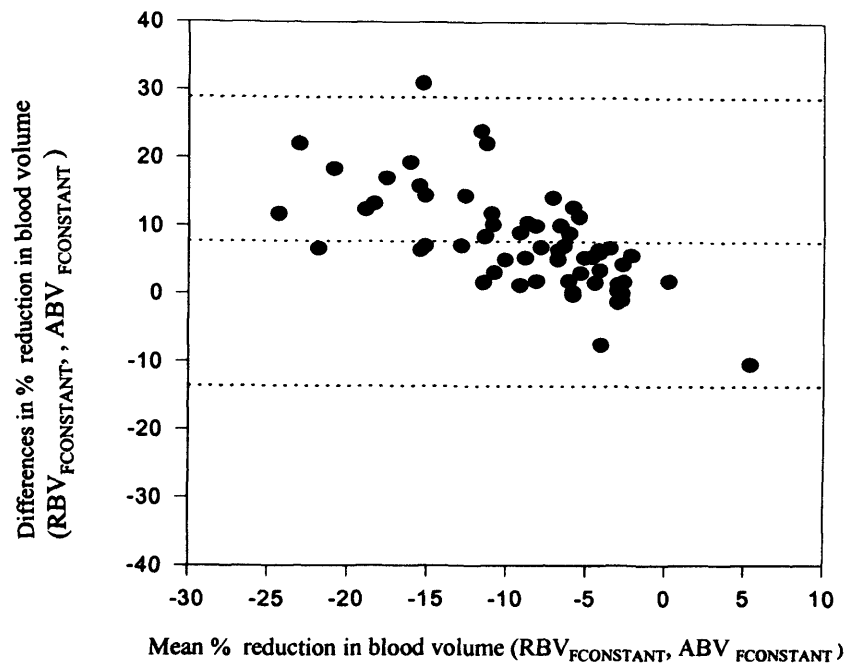


Fig 5a

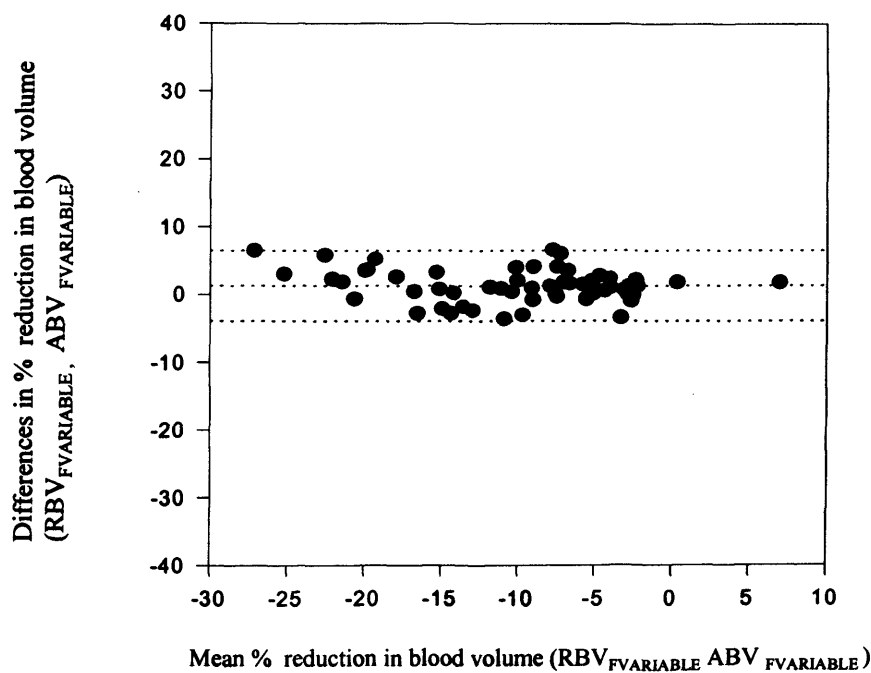


Fig 5b

Figure 5. Bland Altman analysis comparing percentage blood volume changes in 10 HD patients during UF as observed by RBV monitor and absolute blood volume measured by ICG technique (% blood volume reduction during UF between all four measurements; $n = 60$). Reference lines indicate mean difference ± 2 SD. (a) Comparing observed RBV_{BVM} changes and absolute blood volume changes derived from plasma volume, Hct, and Fcell ratio 0.86 ($ABV_{Fconstant}$). (b) Comparing RBV_{BVM} changes and absolute blood volume changes ($ABV_{Fvariable}$) both corrected for Fcell variation. RBV changes underestimate absolute blood volume reduction with constant F cell assumption by $7.7 \pm 10.7\%$ (5a) but by only $1.2 \pm 2.6\%$ when corrected for varying F cell ratio (5b)

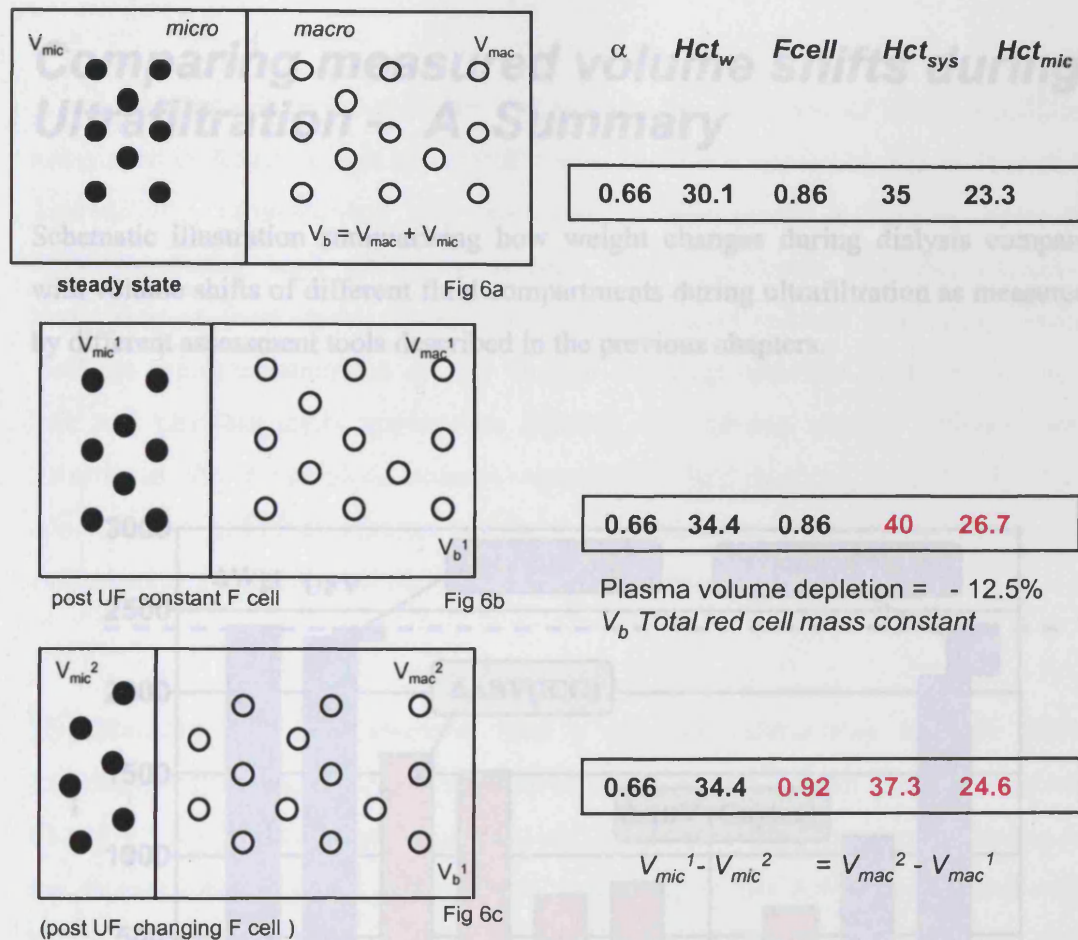


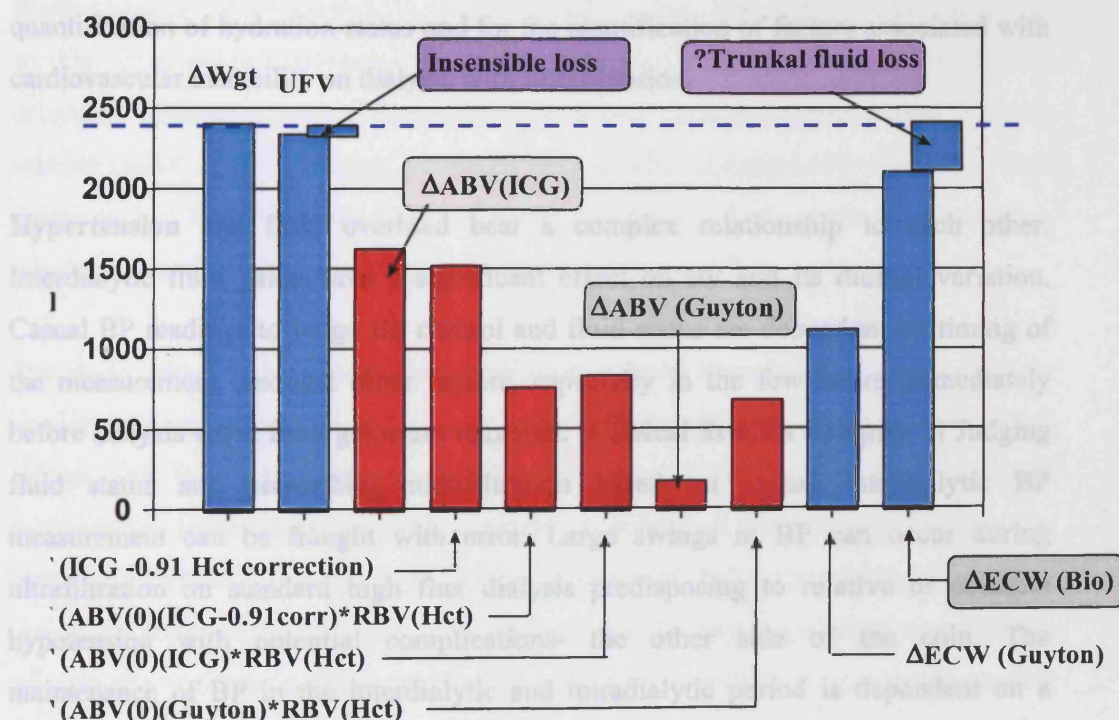
Figure 6 Proposed hypothetical blood volume model demonstrating microvascular volume change (MVC) 6a Steady-state control shows uniform systemic hematocrit (H_{sys}) for the macrocirculation (right compartment V_{mac}) and the microvascular haematocrit H_{mic} for the microcirculation (left compartment V_{mic}) to represent the total red blood cell content in a blood volume V_b and whole body hematocrit (H_w). 6b post UF considers a state of net volume removed $D V_b$ resulting from ultrafiltration and a static relationship between the contracted macro- (V_{mac}^1) and microcirculation (V_{mic}^1) (F cell constant). 6c Identical net blood volume depletion ($D V_b$) with an additional microvascular volume change (V_{mic}^2), resulting in a rise in the Fcell ratio and underestimation of the Hctsys change. It demonstrates a state of redistribution from micro to macrocirculation (macrovascular filling or intravascular refill) during UF. μ (2/3) represents a constant proportional adjustment between the macro and micro hematocrit.

Chapter 10

OVERVIEW

Comparing measured volume shifts during Ultrafiltration – A Summary

Schematic illustration summarising how weight changes during dialysis compare with volume shifts of different fluid compartments during ultrafiltration as measured by different assessment tools described in the previous chapters.



- ΔWgt mean reduction in body weight pre and post dialysis
- ΔUFV volume of ultrafiltrate removed
- $\Delta ABV (ICG)$ mean absolute blood volume reduction measured by ICG
- $\Delta RBV (Hct)$ haematocrit derived from relative blood volume monitor.
- $\Delta ABV (Guyton)$ mean reduction in blood volume predicted by Guyton formula
- $\Delta ECW (Guyton)$ mean reduction in extracellular volume predicted by Guyton
- $\Delta ECW (Bio)$ mean reduction in extracellular volume measured by Bioimpedance
- $\Delta ICG - 0.91 Hct\ corr$ ABV derived using $ABV(ICG) \& F_{cell}$ correction

OVERVIEW

A major aim of this series of studies was to study the dynamics of vascular refilling using short intermittent high rate ultrafiltration boli and observe changes in fluid shift (extracellular, intravascular) and cardiovascular parameters at varying states of hydration during high efficiency dialysis. The hypothesis was that objective monitoring of fluid status during dialysis using blood volume monitoring would facilitate accurate estimation of 'dry weight' and help optimise fluid removal in a safe and physiologically appropriate manner. The clinical studies included here investigated the use of blood volume responses to fluid removal, both for objective quantification of hydration status and for the identification of factors associated with cardiovascular instability on dialysis with ultrafiltration.

Hypertension and fluid overload bear a complex relationship to each other. Interdialytic fluid gains have a significant effect on BP and its diurnal variation. Casual BP readings to judge BP control and fluid status are dependent on timing of the measurement amongst other factors, especially in the few hours immediately before dialysis when fluid gains are maximal. [Clinical Studies Chapter 1] Judging fluid status and prescribing ultrafiltration based on casual interdialytic BP measurement can be fraught with error. Large swings in BP can occur during ultrafiltration on standard high flux dialysis predisposing to relative or absolute hypotension with potential complications- the other side of the coin. The maintenance of BP in the interdialytic and intradialytic period is dependent on a combination of factors i.e. extracellular volume, circulating blood volume and vascular tone, which is intricately regulated by baroreceptor and neurohormonal reflexes. These altered adjusting mechanisms coupled with rapid fluxes in extracellular compartment due to intermittent dialysis lead to blood pressure variations during the dialytic and interdialytic cycle.

The quality of **blood pressure monitoring** depend not only on technical limitations but more commonly on understanding of the user of the technology. Often

inappropriate selection of cuff size or movement artefacts may invalidate the results. Guidelines on the correct methodology of BP measurement, published by various professional societies are often ignored. This is particularly true with respect to the need for a sufficient resting period prior to BP measurements. The specific influence of the timing, measurement method and dialysis frequency on reading accuracy has been insufficiently perceived. The study reported here and other studies have shown that sole reliance on predialysis casual readings is invalid. Consensus on BP targets in haemodialysis is proving very difficult to achieve. This may reflect inadequacies in measurement, monitoring and treatment.

Bioimpedance spectroscopy is highly reproducible and particularly useful in tracking fluid shifts with some parameters particularly useful and more sensitive in detecting changes in hydration status of these patients. Extracellular volume measurements tracked change in body weight with considerable accuracy. This technique has the added advantage of incorporating lean body mass characteristics, which are closely linked to the hydration status of these patients.

The study reported here has demonstrated that **fluid distribution** is abnormal in uremic subjects compared to controls especially in patients with advanced renal failure and those on dialysis. [Clinical Studies Chapter 2] The expanded extracellular compartment is most apparent in advanced uraemia approaching the need for dialysis. This could be a consequence of failing nutrition. Both may precipitate the need for initiating renal replacement therapy. Significant malnutrition is a late phenomenon in advanced renal insufficiency and there is a profound decline in nutritional state just prior to dialysis initiation. Nutritional status is only partially corrected by dialysis despite adequate modelling parameters. Visceral protein markers are less reliable than direct tools in the assessment of nutritional status. Falling nPCR quite early in the course of progressive renal failure may be partly due to mathematical coupling and errors in urine collection. The volume expansion is also only partially corrected by dialysis treatment. Poorly corrected malnutrition may have a significant role in overhydration of dialysis patients. Dry weight adjustments must include nutritional assessment as a core element.

Both blood density and sound speed are closely related to total protein concentration in blood and, as a consequence, to rheologically important parameters of blood. The methods that permit continuous measurement of these properties, the ultrasonic technique, used for measuring relative blood volume changes over a continuous period of time in haemodialysis patients. From the continuous measurement of concentrations during haemodialysis treatment, relative changes in blood volume can be recorded in real time.

The subsequent chapters on **relative blood volume (RBV)** measurement shift focus from hypertension and volume overload to investigate hypotension and approaching dry weight. There is little change in the blood volume profile during a stable supine dialysis session with no UF. The application of UF significantly alters the RBV profile during dialysis. Pattern recognition of these changes yield variable curves primarily influenced by UF prescription with wide inter-patient and intra-patient variability. It was apparent that steeper decay of blood volume curve was more frequently associated with haemodynamic instability. Diagnosis was however retrospective and subjective. **[Clinical Studies Chapter 3]**

The degree of change in RBV per unit volume of ultrafiltrate was higher in patients nearer to their predicted dry weight. **[Clinical Studies Chapter 4]** The critical level of reduction in RBV at which hypotension occurs (RBVcrit) showed a wide inter-individual range, varying from 71 to 98%. No correlation was observed with changes in RBV and BP or the incidence of hypotension. The RBVcrit may also depend on cardiovascular defence mechanisms such as sympathetic drive. The concept of a critical relative blood volume reduction leading to hypotension cannot be sustained.

RBV decay characteristics **[Clinical Studies Chapter 5]** under experimental conditions can detect changes in hydration status and predict instability. They are determined by the plasma refilling capacity presumably driven by hydrostatic forces. The refilling analysis remains intriguing and warrants further investigation and better analysis techniques e.g. refilling rate distribution function with time, which could be constructed for each period of ultrafiltration. The results of the study reported here indicate a 2 pool linear behaviour of the extracellular compartment during short

stress ultrafiltration and highlight the importance of compartmental fluid shifts during UF. The main buffering mechanism for BV stability is the interstitial space.

The ultrafiltration rate has an independent effect on the RBV independent of the hydration state, amount of fluid removed or patient related factors. Cardiovascular response systems are constantly stressed in the inter- and intra-dialytic phase to cope with the fluctuating hydration status. This influences haemodynamic variables such as BP and RBV. For this reason RBV patterns and response will vary according to the dialysis schedule. Further RBV studies on longer treatment duration or more frequent therapies will provide a better insight into the influence of time on RBV and BP changes during dialysis with ultrafiltration. Extracellular compartment volume tracking with simultaneous RBV measurement may help prescribe adequate and safe ultrafiltration regimen for each subject during dialysis.

Clinical Studies Chapter 6 explores how other prescription variables such as dialysate temperature can be used in conjunction with ultrafiltration to allow improved haemodynamic stability.

The technique of dye dilution using indocyanine green was successfully used to investigate changes in absolute plasma and blood volume during dialysis with ultrafiltration, compare them directly with RBV alterations and quantify plasma refill volume. [Clinical Studies Chapters 7-10] It is apparent that RBV alterations during ultrafiltration are induced by intravascular volume depletion and microcirculatory changes within the vasculature, the latter effect more pronounced at the later stages of ultrafiltration nearer to dry weight.

During the RBV studies it was seen that the concentration of blood components varies considerably with ultrafiltration. The alterations in Fcell ratio during ultrafiltration suggest that rapid fluctuations at the macroscopic scale with periods of 5 to 30 seconds are possibly due to heterogeneities at the microscopic scale and to the particular rheological behaviour of the red blood cells at the level of the capillaries and the small blood vessels.

Many of the observations contained in this thesis deserve further study. In the following sections I have attempted to formulate a framework for future work in this area.

1. The Microcirculation

The effect of microcirculatory shifts on cardiac function, humoral control of the microcirculation and therapeutic role of microcirculation in blood volume compensation are little studied. The measurement of concentrations of circulating plasma and blood components at an accessible measuring site may be used to investigate the rheology of blood in the human microvasculature. Study of the mechanisms by which the microcirculation is recruited and ways in which it can be manipulated to improve macrovascular filling and cardiovascular function would be a major advance.

2. Fluid kinetic model

Fluid models will need to be developed to provide more rigorous analysis of blood volume responses. Modelling ultrafiltration requires a degree of understanding of active compensatory mechanisms that is currently lacking. The models require the capacity to modify the ratio of stressed to unstressed volume in the vascular space, to take account of the lymphatic shift and to allow some form of active refilling. Changes in blood flow distribution to the different sections of the microvasculature will influence the filtration coefficient and determine plasma refill - an effect not considered in passive compartment models. Such models could provide the basis for a parameter based adaptive control strategy to remove fluid in a more physiological manner.

3. Effects of recirculation

The extracorporeal circuit may also have a role to play in the blood volume changes noted. It is appreciated that transport delays are introduced as ultrafiltered blood is translated into a blood volume change via the patient. There is the mixing effect caused by cardiopulmonary recirculation and the possibility of large artefactual

changes in blood volume resulting from access recirculation. Recirculation of ultrafiltered blood back to the blood volume monitor without passing through the patient's systemic circulation can cause the apparent relative blood volume to change more rapidly during ultrafiltration. The effect of cardiopulmonary recirculation on blood volume changes has not been fully explored especially with respect to its influence on maximal refilling rate. In the current data sets the effect of access recirculation could be eliminated by use of a temperature control module and monitoring of the transport delay between each UF transition and the slope change in relative blood volume. More rigorous analysis of recirculation effects deserve further attention for fluid kinetic modelling. Suitable corrections could be applied to blood volume data.

4. Interaction between variables

The current research focussed on relationships between blood volumes, blood pressure and bioimpedance changes during dialysis. The major factors governing stability are blood volume, plasma refill, temperature and vascular tone. There are a large number of patient related variables relating to hydration status such as vascular compliance, plasma volume expansion, changes in capillary pressure and regional blood flow redistribution. The effect of these may be crucial in determining haemodynamic alterations. More extensive studies are required to evaluate the changes in cardiac output, antihypertensive medications, pulse rate variability, the role of the intracellular space during refill, dialysate conductivity and the role of various comorbidities. In all patients, HD with UF increase urea rebound when compared with isovolaemic HD. The regional blood flow model offers a physiologic interpretation of this observation in which hypovolaemic compensation reduces peripheral blood flow thereby increasing peripheral urea sequestration. From the clinical point of view it follows that two main aspects of HD adequacy, clearance and UF, are not independent. The relationship becomes more important as treatment times are reduced and UF rates are increased. Switching from constant to variable UF modes such as using high initial and subsequently decreasing UF rates is likely to have positive effects both on UF-induced blood volume reduction and on dialysis efficiency. The effect of vasoactive substances, hypoxemia, plasma osmolality, body position, eating etc has not been examined. Effect of postural manoeuvres on blood

volume changes will be an important consideration in the design of feedback control systems and much further study will be required to characterise the effects of changing posture in different haemodynamic states. A model of the venous and arterial trees of the peripheral and central circulation might facilitate the modelling of temperature effects since the effect is predominantly through shunt vessels.

5. Tolerance to ultrafiltration in different treatment modalities.

The effects of dialysis and ultrafiltration cannot be separated in these studies. It would be instructive to compare patient responses to both isolated UF and combined UF and dialysis in a cross-over study. Eliminating dialysis process offers advantages for bioimpedance measurements since electrolyte composition can be maintained constant. Sodium variation during a period of isovolaemia may be able to tease out the specific effects of electrolyte variation on bioimpedance measurements. The effect of modifying dialysate buffer on blood volume stability needs further exploration to minimise haemodynamic compromise. [279]

6. Absolute blood volumes (ABV) derivation

The rate of change of slope of raw relative blood volume data may be used to derive absolute blood volume in real time in a non-invasive manner. Although slope determination is a relatively straightforward operation, different methods for absolute blood volume calculation may be developed based on algorithms. Two such algorithms using localised RBV calculation depend on local blood water (BW_0) information. The remaining three algorithms make use of the rate of change of blood water [$d(BW)/dt$ data]. [280] Preliminary analysis has suggested that using raw ABV estimations the mean difference between the ultrafiltration algorithm and ICG is approx. 400 ml. i.e. the ABV algorithm underestimates the ABV measured by Indocyanine (ICG) method. Importantly the deviations about the mean are *always* patient specific i.e. the algorithm systematically under- or over-estimates the ABV determined via ICG, perhaps because different methods see different compartments or distribution volumes. Nevertheless since the error is systematic in patients, it allows some rules to be defined for slope evaluation. The capacity to derive an absolute parameter of the vascular compartment during dialysis in a simple manner

will be extremely useful in addressing the issue of vascular stability and hydration status of these patients. Absolute blood volume derivation using refined algorithms is a potentially non-invasive way of monitoring fluid status during ultrafiltration. The use of ICG monitoring in a non-invasive manner also deserves further exploration for direct and reliable quantification of plasma and blood volumes.

7. Finally, data acquisition and processing involved in these studies were extremely time consuming and a major constraint in this research. For further analysis of such data sets it is recommended that the data be first imported into database tables to expedite progress. A more robust integrated data processing system would enable high quality studies in this field. The data and studies in this thesis have been largely cross sectional. While this is useful in obtaining ranges of parameters, longitudinal studies are necessary to realise full potential of many of these ideas.

Conclusion

An understanding of the physiologic fluid shifts in the vascular space during fluid removal on dialysis and more objective measures of the fluid status is necessary for accurate estimation of dry weight and safe fluid removal on dialysis. The availability of modern dialysis machines equipped with various devices to monitor the patient and the dialysis treatment hold promise for better understanding and future improvements in ultrafiltration prescription. The major limiting factor is the patient response pattern to ultrafiltration, hence individualising prescription may help. This requires a system, which can characterise the response and feedback instantaneously. I hope that the work described here may contribute to this goal.

Appendix 1

A typical real time data acquisition file obtained during a dialysis session (Acqui download)

Time	BVM_R	BVM_H	BP_SY	BP_DIA	BP_PUI	P_Ven	P_Art	Qb	Cond	UFV	UFV	UF_Tim	Qd	BTM_T	BTM_T	BTM_J
Time	BVM_R	BVM_H	BP_SY	BP_DIA	BP_PUI	P_Ven	P_Art	Qb	Cond	UFV	UFV	UF_Tim	Qd	BTM_T	BTM_T	BTM_J
5	100.18	30.71														
6	100.18	30.71														
11	100.37	30.62														
14	100.36	30.62														
17	100.36	30.63														
21	100.4	30.61														
24	100.36	30.63														
27	100.32	30.65														
30	100.37	30.62														
33	100.33	30.64														
36	100.33	30.64														
39	100.51	30.56														
42	100.43	30.6														
45	100.29	30.66														
48	100.51	30.56														
51	100.51	30.56														
54	100.53	30.55														
57	100.48	30.57														
60	100.52	30.56														
63	100.48	30.58														
66	100.51	30.56														
69	100.54	30.55														
72	100.72	30.48														
75	100.55	30.48														
78	100.61	30.51														
81	100.61	30.51														
84	100.61	30.51														
87	100.61	30.51														
90	100.61	30.51														
93	100.61	30.51														
96	100.61	30.51														
99	100.61	30.51														
102	100.61	30.51														
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123	100.61	30.51														
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159	100.61	30.51														
162	100.61	30.51														
165	100.61	30.51														
168	100.61	30.51														
171	100.61	30.51														
174	100.61	30.51														
177	100.61	30.51														
180	100.61	30.51														

Appendix 2

Steps in the derivation of relative blood volume parameters

1. Slope evaluation 2. Cumulative area 3. Refill divergence

Slope Evaluation

PROCEDURE

Data acquisition file obtained

Run cut file program (on visual basic) to create .cut uf files

Filter data

Select UF slabs from each bolus to avoid uf delays

1. Start at bvm ref where the data continues to decrease only
2. End at the lowest bvm after end of uf
3. check that uf rates are more or less constant
4. delete data points that are at lower uf rates
5. ensure the volume is equal and time intervals at 3 secs

Copy each data slab to the cum area template files for 0,30,60,90,120 files

Read slope,start rbv, and net slope deviation

Repeat this for each UF slab

Slope evaluation critically depends on

- precision of data points
- signal noise variation
- data filter enhances the above
- artefactual changes induced by alarms and posture variation
- break in data signal due to hypo,saline,ufstart and stop
- accurate and constant uf rates
- d RBV magnitude (high values would increase net deviation)
- UF delay
- recirculatory effects
- manual errors

Initial slope was determined from 90 secs of data points at different segments of the initial 2 mins of the rbv uf data

at the start of uf decay

30 secs from uf decay

60 secs from uf decay

90 secs from uf decay

120 mins from uf decay

A consistent and significant trend was observed for 60 secs UF slope. This correlated with the distance from dry weight $r^2 = 0.64$. Predictive value may be possible at differences of 500ml of hydration status.

The effect of UF on the blood volume profiles was significant. The curves were defined in another way. Using the first min BV decay we obtained linear regression slopes and calculated the deviation away from linearity. A linear regression slope was drawn from linear BV decay. This patient who became hypotensive at the third step during progressive UF, with equal volume showed smaller deviation from initial regression which near dryness becomes disappearingly small. We called this refill divergence. The same phenomenon is depicted in another patient. We found significant differences in divergence between the pt near and far from dry weight. When measured by AUC assessment. This can be done in a more sophisticated manner by a method of integrative function.

Cumulative area analysis

Linear/ Refill divergence

Using high resolution data, the deviation of BV decay curve away from a linear decay predicted from an initial 1 min slope evaluated 60secs away from onset of UF can be used to estimate the liner divergence :

Steps of analysis

- data acquisition
- data filter function
- determination of slope and predicted linear decay
- refill divergence = {cum. UF RBV area - cum. linear RBV area}

Refill divergence estimation (Limitations)

- very sensitive to correct evaluation of slopes
- resolution of the BVM device
- UF delay
- accurate UF rates
- data artefacts (breathing, machine alarms, posture

(green shaded areas locked and automatically derived using algorithm and data from nonshaded areas)

[illegible]

2575	7725	128.75	96.61	96.55296 3004 1760	7856.72	96.54	7856.30	0.42	2.3
2576	7728	128.8	96.47	96.48753 3004 1762	8146.18	96.50	8145.80	0.38	2.35
2577	7731	128.85	96.26	96.42537 3004 1765	8435.46	96.47	8435.20	0.26	2.4
2578	7734	128.9	96.33	96.37213 3004 1767	8724.57	96.43	8724.50	0.08	2.45
2579	7737	128.95	96.4	96.32284 3004 1770	9013.54	96.40	9013.69	-0.14	2.5
2580	7740	129	96.29	96.27052 3004 1772	9302.35	96.36	9302.77	-0.42	2.55
2581	7743	129.05	96.06	96.21919 3004 1775	9591.01	96.33	9591.75	-0.74	2.6
2582	7746	129.1	96.14	96.17549 3004 1777	9879.54	96.29	9880.63	-1.09	2.65
2583	7749	129.15	96.28	96.13371 3004 1780	10167.94	96.26	10169.40	-1.46	2.7
2584	7752	129.2	96.1	96.08572 3004 1783	10456.20	96.22	10458.06	-1.87	2.75
2585	7755	129.25	95.97	96.03524 3004 1785	10744.30	96.19	10746.62	-2.32	2.8
2586	7758	129.3	95.88	95.98991 3004 1788	11032.27	96.15	11035.08	-2.81	2.85
2587	7761	129.35	95.88	95.95276 3004 1790	11320.13	96.12	11323.43	-3.30	2.9
2588	7764	129.4	95.85	95.92311 3004 1793	11607.90	96.08	11611.68	-3.78	2.95
2589	7767	129.45	95.82	95.9003 3004 1795	11895.60	96.05	11899.82	-4.22	3
2590	7770	129.5	95.84	95.88382 3004 1798	12183.25	96.01	12187.85	-4.60	3.05
2591	7773	129.55	95.81	95.8726 3004 1800	12470.87	95.98	12475.78	-4.91	3.1
2592	7776	129.6	96.08	95.86023 3004 1803	12758.45	95.94	12763.61	-5.16	3.15
2593	7779	129.65	95.85	95.839 3004 1805	13045.97	95.91	13051.33	-5.36	3.2
2594	7782	129.7	95.835	95.81171 3004 1807	13333.40	95.87	13338.94	-5.54	3.25
2595	7785	129.75	95.82	95.78223 3004 1809	13620.75	95.84	13626.46	-5.71	3.3
2596	7788	129.8	95.71	95.75145 3004 1811	13908.00	95.80	13913.86	-5.86	3.35
2597	7791	129.85	95.69	95.72243 3004 1814	14195.17	95.77	14201.16	-5.99	3.4
2598	7794	129.9	95.59	95.69832 3004 1816	14482.27	95.73	14488.36	-6.09	3.45
2599	7797	129.95	95.66	95.68036 3004 1818	14769.31	95.70	14775.45	-6.14	3.5
2600	7800	130	95.9	95.66032 3004 1820	15056.29	95.66	15062.44	-6.15	3.55
2601	7803	130.05	95.62	95.63004 3004 1823	15343.18	95.63	15349.32	-6.14	3.6
2602	7806	130.1	95.64	95.59367 3004 1825	15629.96	95.59	15636.09	-6.14	3.65
2603	7809	130.15	95.76	95.55063 3004 1828	15916.61	95.56	15922.77	-6.16	3.7
2604	7812	130.2	95.57	95.49525 3004 1830	16203.10	95.52	16209.33	-6.24	3.75
2605	7815	130.25	95.38	95.43289 3004 1833	16489.40	95.49	16495.79	-6.40	3.8
2606	7818	130.3	95.17	95.37594 3004 1835	16775.52	95.45	16782.15	-6.63	3.85
2607	7821	130.35	95.27	95.33099 3004 1838	17061.52	95.42	17068.40	-6.89	3.9
2608	7824	130.4	95.33	95.29269 3004 1840	17347.39	95.38	17354.55	-7.16	3.95
2609	7827	130.45	95.18	95.25579 3004 1843	17633.16	95.35	17640.59	-7.43	4
2610	7830	130.5	95.31	95.21944 3004 1846	17918.82	95.31	17926.53	-7.71	4.05
2611	7833	130.55	95.11	95.18233 3004 1850	18204.37	95.28	18212.36	-7.99	4.1
2612	7836	130.6	95.18	95.14568 3004 1853	18489.80	95.24	18498.09	-8.28	4.15
2613	7839	130.65	95.08	95.10978 3004 1857	18775.13	95.21	18783.71	-8.58	4.2
2614	7842	130.7	94.97	95.0769 3004 1860	19060.36	95.17	19069.23	-8.86	4.25
2615	7845	130.75	94.86	95.05402 3004 1862	19345.53	95.14	19354.64	-9.11	4.3
2616	7848	130.8	94.93	95.04505 3004 1865	19630.66	95.10	19639.95	-9.29	4.35
2617	7851	130.85	95.035	95.04803 3004 1867	19915.80	95.07	19925.15	-9.35	4.4
2618	7854	130.9	95.14	95.04803 3004 1869	20200.94	95.03	20210.25	-9.30	4.45
2619	7857	130.95	95.19	95.04272 3004 1871	20486.07	95.00	20495.24	-9.17	4.5
2620	7860	131	95.16	95.02552 3004 1874	20771.15	94.96	20780.13	-8.98	4.55
2621	7863	131.05	94.93	95.00041 3004 1876	21056.15	94.93	21064.91	-8.76	4.6
2622	7866	131.1	95.1	94.97254 3004 1878	21341.07	94.89	21349.59	-8.52	4.65
2623	7869	131.15	95.04	94.93813 3004 1880	21625.88	94.86	21634.16	-8.28	4.7
2624	7872	131.2	94.92	94.89558 3004 1883	21910.57	94.82	21918.63	-8.06	4.75
2625	7875	131.25	94.65	94.85426 3004 1885	22195.13	94.79	22202.99	-7.86	4.8

2626	7878	131.3	94.74	94.82362 3004 1888	22479.60	34.75	22487.25	-7.65	4.85
2627	7881	131.35	94.85	94.80034 3004 1890	22764.00	34.72	22771.40	-7.40	4.9
2628	7884	131.4	94.77	94.77684 3004 1893	23048.33	34.68	23055.45	-7.12	4.95
2629	7887	131.45	94.77	94.76211 3004 1895	23332.59	34.65	23339.39	-6.80	5
2630	7890	131.5	94.77	94.72571 3004 1898	23616.77	34.61	23623.23	-6.46	5.05
2631	7893	131.55	94.77	94.69516 3004 1900	23900.85	34.58	23906.96	-6.11	5.1
2632	7896	131.6	94.72	94.65878 3004 1903	24184.83	34.54	24190.59	-5.76	5.15
2633	7899	131.65	94.59	94.61856 3004 1905	24468.68	34.51	24474.12	-5.43	5.2
2634	7902	131.7	94.66	94.57632 3004 1908	24752.41	34.47	24757.53	-5.12	5.25
2635	7905	131.75	94.66	94.52789 3004 1910	25036.00	34.44	25040.85	-4.85	5.3
2636	7908	131.8	94.45	94.47202 3004 1913	25319.41	34.40	25324.06	-4.64	5.35
2637	7911	131.85	94.36	94.4154 3004 1915	25602.66	34.37	25607.16	-4.50	5.4
2638	7914	131.9	94.29	94.36348 3004 1918	25885.75	34.33	25890.16	-4.41	5.45
2639	7917	131.95	94.24	94.31796 3004 1920	26168.70	34.30	26173.05	-4.35	5.5
2640	7920	132	94.28	94.27762 3004 1923	26451.54	34.26	26455.84	-4.30	5.55
2641	7923	132.05	94.24	94.23894 3004 1925	26734.25	34.23	26738.52	-4.27	5.6
2642	7926	132.1	94.14	94.20155 3004 1928	27016.86	34.19	27021.10	-4.25	5.65
2643	7929	132.15	94.1	94.16852 3004 1930	27299.36	34.16	27303.58	-4.21	5.7
2644	7932	132.2	94.07	94.14165 3004 1932	27581.79	34.12	27585.95	-4.16	5.75
2645	7935	132.25	94.07	94.12079 3004 1934	27864.15	34.09	27868.21	-4.06	5.8
2646	7938	132.3	94.03	94.10559 3004 1937	28146.47	34.05	28150.37	-3.90	5.85
2647	7941	132.35	93.98	94.0977 3004 1939	28428.76	34.02	28432.42	-3.66	5.9
2648	7944	132.4	93.94	94.10058 3004 1941	28711.06	33.98	28714.37	-3.31	5.95
2649	7947	132.45	94.09	94.11403 3004 1943	28993.40	33.95	28996.22	-2.81	6
2650	7950	132.5	94.28	94.12878 3004 1946	29275.79	33.91	29277.96	-2.17	6.05
2651	7953	132.55	94.34	94.13243 3004 1948	29558.19	33.88	29559.59	-1.40	6.1
2652	7956	132.6	94.27	94.11971 3004 1951	29840.55	33.84	29841.12	-0.57	6.15
2653	7959	132.65	94.25	94.09177 3004 1953	30122.82	33.81	30122.55	0.28	6.2
2654	7962	132.7	94.05	94.05323 3004 1956	30404.98	33.77	30403.86	1.12	6.25
2655	7965	132.75	93.94	94.01289 3004 1958	30687.02	33.74	30685.08	1.94	6.3
2656	7968	132.8	93.87	93.97844 3004 1961	30968.96	33.70	30966.19	2.77	6.35
2657	7971	132.85	93.8	93.95412 3004 1963	31250.82	33.67	31247.19	3.62	6.4
2658	7974	132.9	93.96	93.93883 3004 1966	31532.63	33.63	31528.09	4.54	6.45
2659	7977	132.95	94.01	93.92413 3004 1968	31814.41	33.60	31808.89	5.52	6.5
2660	7980	133	93.96	93.90373 3004 1971	32096.12	33.56	32089.58	6.54	6.55
2661	7983	133.05	93.935	93.87749 3004 1973	32377.75	33.53	32370.16	7.59	6.6
2662	7986	133.1	93.91	93.84588 3004 1976	32659.29	33.49	32650.64	8.64	6.65
2663	7989	133.15	93.77	93.81094 3004 1980	32940.72	33.46	32931.02	9.70	6.7
2664	7992	133.2	93.82	93.7754 3004 1983	33222.05	33.42	33211.29	10.76	6.75
2665	7995	133.25	93.74	93.73872 3004 1987	33503.26	33.39	33491.45	11.81	6.8
2666	7998	133.3	93.58	93.70379 3004 1990	33784.38	33.35	33771.52	12.86	6.85
2667	8001	133.35	93.62	93.67576 3004 1992	34065.40	33.32	34051.47	13.93	6.9
2668	8004	133.4	93.45	93.65784 3004 1995	34346.38	33.28	34331.32	15.05	6.95
2669	8007	133.45	93.45	93.6554 3004 1997	34627.34	33.25	34611.07	16.28	7
2670	8010	133.5	93.76	93.6649 3004 1999	34908.34	33.21	34890.71	17.63	7.05
2671	8013	133.55	93.76	93.67276 3004 2002	35189.36	33.18	35170.24	19.11	7.1
2672	8016	133.6	93.76	93.67245 3004 2004	35470.37	33.14	35449.67	20.70	7.15
2673	8019	133.65	93.76	93.66396 3004 2007	35751.36	33.11	35729.00	22.36	7.2
2674	8022	133.7	93.67	93.64859 3004 2009	36032.31	33.07	36008.22	24.09	7.25
2675	8025	133.75	93.63	93.63004 3004 2011	36313.20	33.04	36287.34	25.86	7.3
2676	8028	133.8	93.67	93.60963 3004 2014	36594.03	33.00	36566.35	27.68	7.35

2677	8031	133.85	93.63	93.58544 3004 2016	36874.79	92.97	36845.25	29.53	7.4
2678	8034	133.9	93.5	93.55918 3004 2018	37155.46	92.93	37124.05	31.41	7.45
2679	8037	133.95	93.45	93.53659 3004 2021	37436.07	92.90	37402.75	33.32	7.5
2680	8040	134	93.66	93.51601 3004 2023	37716.62	92.86	37681.34	35.28	7.55
2681	8043	134.05	93.48	93.49109 3004 2026	37997.09	92.83	37959.83	37.27	7.6
2682	8046	134.1	93.44	93.46393 3004 2028	38277.49	92.79	38238.21	39.28	7.65
2683	8049	134.15	93.47	93.43736 3004 2031	38557.80	92.76	38516.49	41.31	7.7
2684	8052	134.2	93.58	93.40586 3004 2033	38838.02	92.72	38794.66	43.36	7.75
2685	8055	134.25	93.48	93.36299 3004 2036	39118.10	92.69	39072.72	45.38	7.8
2686	8058	134.3	93.26	93.31199 3004 2038	39398.04	92.65	39350.69	47.35	7.85
2687	8061	134.35	93.26	93.2607 3004 2041	39677.82	92.62	39628.54	49.28	7.9
2688	8064	134.4	93.26	93.20947 3004 2043	39957.45	92.58	39906.29	51.16	7.95
2689	8067	134.45	93.07	93.15796 3004 2046	40236.92	92.55	40183.94	52.98	8
2690	8070	134.5	92.9	93.1142 3004 2048	40516.27	92.51	40461.48	54.78	8.05
2691	8073	134.55	93.07	93.08252 3004 2051	40795.51	92.48	40738.92	56.60	8.1
2692	8076	134.6	93.01	93.05737 3004 2053	41074.69	92.44	41016.25	58.44	8.15
2693	8079	134.65	92.98	93.03596 3004 2055	41353.79	92.41	41293.48	60.32	8.2
2694	8082	134.7	92.95	93.01979 3004 2058	41632.85	92.37	41570.60	62.25	8.25
2695	8085	134.75	92.95	93.00943 3004 2060	41911.88	92.34	41847.62	64.26	8.3
2696	8088	134.8	93.03	93.00276 3004 2062	42190.89	92.30	42124.53	66.36	8.35
2697	8091	134.85	93.05	92.99495 3004 2065	42469.88	92.27	42401.34	68.54	8.4
2698	8094	134.9	93.06	92.98222 3004 2067	42748.82	92.23	42678.04	70.78	8.45
2699	8097	134.95	92.97	92.96455 3004 2070	43027.72	92.20	42954.64	73.08	8.5
2700	8100	135	92.87	92.94661 3004 2072	43306.56	92.16	43231.13	75.43	8.55
2701	8103	135.05	92.89	92.93301 3004 2074	43585.35	92.13	43507.52	77.84	8.6
2702	8106	135.1	93	92.92134 3004 2077	43864.12	92.09	43783.80	80.32	8.65
2703	8109	135.15	92.94	92.90628 3004 2079	44142.84	92.06	44059.98	82.86	8.7
2704	8112	135.2	92.88	92.88807 3004 2081	44421.50	92.02	44336.05	85.45	8.75
2705	8115	135.25	92.79	92.87132 3004 2084	44700.12	91.99	44612.02	88.10	8.8
2706	8118	135.3	92.96	92.85608 3004 2086	44978.68	91.95	44887.88	90.80	8.85
2707	8121	135.35	92.97	92.83486 3004 2089	45257.19	91.92	45163.64	93.55	8.9
2708	8124	135.4	92.83	92.80452 3004 2091	45535.60	91.88	45439.29	96.31	8.95
2709	8127	135.45	92.83	92.76852 3004 2094	45813.91	91.85	45714.84	99.07	9
2710	8130	135.5	92.8	92.72748 3004 2096	46092.09	91.81	45990.28	101.81	9.05
2711	8133	135.55	92.59	92.68385 3004 2099	46370.14	91.78	46265.62	104.52	9.1
2712	8136	135.6	92.55	92.64503 3004 2101	46648.08	91.74	46540.85	107.22	9.15
2713	8139	135.65	92.58501	92.61333 3004 2104	46925.92	91.71	46815.98	109.93	9.2
2714	8142	135.7	92.62	92.58428 3004 2106	47203.67	91.67	47091.01	112.66	9.25
2715	8145	135.75	92.53	92.55481 3004 2109	47481.33	91.64	47365.92	115.41	9.3
2716	8148	135.8	92.51	92.52602 3004 2111	47758.91	91.60	47640.74	118.17	9.35
2717	8151	135.85	92.53	92.4978 3004 2113	48036.41	91.57	47915.45	120.96	9.4
2718	8154	135.9	92.46333	92.46857 3004 2115	48313.81	91.53	48190.05	123.76	9.45
2719	8157	135.95	92.39667	92.43985 3004 2118	48591.13	91.50	48464.55	126.58	9.5
2720	8160	136	92.33	92.41515 3004 2120	48868.38	91.46	48738.94	129.43	9.55
2721	8163	136.05	92.42	92.39476 3004 2122	49145.56	91.43	49013.23	132.33	9.6
2722	8166	136.1	92.29	92.37715 3004 2124	49422.69	91.39	49287.41	135.28	9.65
2723	8169	136.15	92.28	92.36488 3004 2127	49699.79	91.36	49561.49	138.29	9.7
2724	8172	136.2	92.37	92.35822 3004 2129	49976.86	91.32	49835.47	141.39	9.75
2725	8175	136.25	92.51	92.34927 3004 2132	50253.91	91.29	50109.34	144.57	9.8
2726	8178	136.3	92.4	92.33115 3004 2136	50530.90	91.25	50383.10	147.80	9.85
2727	8181	136.35	92.4	92.304 3004 2139	50807.81	91.22	50656.76	151.05	9.9

2728	8184	136.4	92.35	92.26904	3004	2143	51084.62	91.18	50930.31	154.31	9.95
2729	8187	136.45	92.22	92.22839	3004	2146	51361.31	91.15	51203.76	157.54	10
2730	8190	136.5	92.09	92.18852	3004	2149	51637.87	91.11	51477.11	160.76	10.05
2731	8193	136.55	91.95	92.1581	3004	2151	51914.35	91.08	51750.35	164.00	10.1
2732	8196	136.6	92.06	92.14128	3004	2154	52190.77	91.04	52023.48	167.29	10.15
2733	8199	136.65	92.14	92.13277	3004	2156	52467.17	91.01	52296.51	170.66	10.2
2734	8202	136.7	92.12	92.1259	0	2159	52743.55	90.97	52569.44	174.11	10.25

Patient's test for all test prior to start

Needle in Sampled when in Red , orange, brown bottles

Prepare dye, Measure Discharge, recordation

Draw dye and Flush line ready

Weigh syringe

Keep 10ml of patients Blood from V ready for flush.

Dye injected in V and Flush line with blood.

Weigh syringe

3 min pause

Blood sampling

Wash out dead space in A and push back in the V

Time of wash noted and orange tube sample taken

Wait for 20min before next

7 washouts in total

Connect patient to HD3 weight connection

Start dialysate collection

Connect BVM , BIO, comp and start Data acquisition system

20 mins later, Machine in bypass, Check BVM recording

Additional red ,orange and brown from A

Draw dye and Flush line ready with blood

Weigh syringe

Inject dye in bubble trap rapidly

Weigh syringe

Sample from A at 3min and every 30 secs

Restart dialysis

Obtain a small sample of dialysate in tube for absorbance

Measure bio, Check BVM and dialysate tank

1 hr later Machine in bypass, Check BVM

Additional red ,orange and brown bottles from A

Draw dye and flush line ready

Weigh syringe

Inject dye in bubble trap rapidly and Flush

Weigh syringe

Sample from A at 3min and every 30 mins

Restart dialysis. Obtain a small sample of dialysate in tube for absorbance

Equipment used

- BVM, Data acquisition system
- Scale and timer
- Syringe 2ml (3)
- Orange tubes (15 labelled)
- Red tubes (3) Brown tubes (3) Black tubes (3)
- Centrifuge – Spin all orange samples for 30 mins 2000rpm
- For assay 30ml syringes (2) 2ml syringes (11) 10ml (3)
- microlitre pipettes
- Lab Spectrophotometer

Appendix 3

Detailed experimental steps during a study over a single dialysis session in phase I and II

Patients rest for 20 min prior to start
Needle in Samples taken in Red , orange, brown bottles
Prepare dye, Measure Bioimpedance , recirculation

**Draw dye and flush line ready
Weigh syringe**

Keep 10ml of patients blood from V ready for flush.
Dye injected in V and Flush line with blood.
Weigh syringe
3 min pause
Blood sampling
Wash out dead space in A and push back in the V
Time of wash noted and orange tube sample taken
Wait for 30sec before next
7 washouts in total

Connect patient to HD straight connection
Start dialysate collection
Connect BVM , BIO, temp and start Data acquisition system
20 mins later, Machine in bypass ,Check BVM recording
Additional red ,orange and brown from A

**Draw dye and flush line ready with blood
Weigh syringe**

Inject dye in bubble trap rapidly
Weigh syringe
Sample from A at 3min and every 30 secs
Restart dialysis
Obtain a small sample of dialysate in tube for absorbance
Measure bio, Check BVM and dialysate tank
1 hr later Machine in bypass, Check BVM
Additional red ,orange and brown bottles from A

Draw dye and flush line ready

Weigh syringe
Inject dye in bubble trap rapidly and flush
Weigh syringe
Sample from A at 3min and every 30 secs
Restart dialysis, Obtain a small sample of dialysate in tube for absorbance

Equipments used

- BVM Data acquisition system
- Scale and timer ,
- Syringe 2ml (3)
- Orange tubes (35 labelled)
- Red tubes (3) Brown tubes (3) Black tubes (3)
- Centrifuge -- Spin all orange samples for 10 min 2000/min
- For assay 30 ml syringes (3) 2ml syringes (21) 10ml (2)
- microlitre pipettes
- Lab Spectrophotometer

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Appendix 4

The following formulae have been used for plasma volume, blood volume and body surface area.

Plasma volume (dye) (dye dilution)	$\frac{\text{dye infused (mg)}}{\text{plasma dye concentration (mg / l)}}$Eqn 1
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Blood volume [247,248] 2	$\frac{\text{Plasma volume (dye)}}{1 - (\text{Hct}_{\text{sys}}) 0.86}$Eqn
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Body surface area [249] 3	$71.84 \times \text{Body weight}^{0.425} \times \text{Height}^{0.725}$...Eqn
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Formulae for deriving blood volume

Guyton formula [250] 4	$5 / 70 \times \text{body weight}$Eqn
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Hidalgo et al (1962)[251]	$0.367 \times \text{Height}^3 + 0.0322 \times \text{Weight} + 0.60$Eqn 5
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Allen et al (1956)[251] 0.0417 (0.414F) x Height ³ + 0.0450 (0.0328F) x Weight - 0.03	$(F = \text{female})$Eqn 6
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Baker et al (1957)[251]	$0.0193 \times \text{Height}^{0.725} \times \text{Weight}^{0.425}$Eqn 7
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Appendix 5

DOSE AMENDMENT AFTER PHASE I :

Dose of Indocyanine green for a single plasma volume estimate:

Dose of dye 5 mg

change to :

Dose of dye 10 mg (≤ 75 kg body weight)
 15mg (> 75 kg body weight)

Rationale for amendment

The dose of the dye in the protocol for a single plasma volume estimate was based on the minimum used in patients on haemodialysis for cardiac output estimation in previous studies. However for plasma volume estimation , one needs to adjust the dose for the body weight to obtain adequate plasma distribution of the dye. The recommendation in the literature is 0.2 mg / kg body weight indocyanine green (max total dose upto 2 mg/kg body weight) for blood volume studies and has been used in doses varying from 10-20 mg per estimate.

In a pilot study at the Lister hospital, 10 mg was found to be sufficient minimum dose required for a reliable estimate of plasma volume in a 65kg man at normohydration while 5 mg achieved inadequate concentrations in the blood. The 10 mg dose has also been used on dialysis population in other centres for the same purpose. Patients on haemodialysis have relatively large blood volumes predialysis due to varying degrees of overhydration. The initial protocol did not recognise the adjustment necessary for this variation in body weight and volume of distribution to obtain reliable estimates.

Based on the pilot study and supported by evidence in the literature, the amendment of the dose to 10mg (≤ 75 kg b.w.) and 15mg (> 75 kg b.w.) with the maximum total dose used per subject for both phases of the study well within the recommended maximum limits is justified.

Appendix 6

Initial Pilot ICG calibration experiments and methodology on two subjects :

Patient 1 JM

Hct: 23%, Alb: 32g/l

Sample pre dialysis

Patient 2 HH

Hct: 38.2%, Alb: 36g/l

Sample mid dialysis

Methods:

25mg of ICG was mixed with 10 ml of solvent to produce ICG solution of 2.5mg/ml. Approx. 7ml of distilled water was withdrawn into a syringe. The mass of the syringe was weighed. The distilled water was then added to a glass bottle and the syringe re-weighed to obtain the volume of distilled water introduced. Approx. 1ml of neat ICG solution was with drawn into a 2 ml syringe. The syringe was weighed before and after the contents has been added to the bottle contained the distilled water. The bottle was mixed and set aside.

Approx. 1ml of plasma was withdrawn into a 2 ml syringe and weight. The contents were injected into a 1ml spectrophotometer cuvette and the syringe was re-weight to obtain the exact volume delivered. The cuvette was placed in the spectrophotometer and zeroed using a fixed wavelength of 805 nm. 10uL of calibration fluid was added to the cuvette. The sample was scanned to confirm that the peak absorption occurred at 805 nm. The absorbance of the plasma containing the ICG calibration fluid in the cuvette was measured. In order to reduce measurement errors the cuvette was removed from the spectrophotometer agitated, replaced and re-measured. This measurement procedure was repeated six times and the readings were averaged.

Dilute calibration fluid was prepared with an identical procedure, using 30 ml of distilled water. In order to produce a range of ICG concentrations, 5 x 10uL aliquots of calibration fluid were added to the plasma sample. Measurements were repeated six times as before at each concentration increment.

- **Concentrated ICG calibration fluid (2 point)**

Mass syringe ICG neat, pre: 4.276g
 Mass syringe ICG neat, post: 3.281g
 Volume ICG neat: 0.995 ml

Mass syringe distilled water filled to approx. 10ml: 13.757g
 Mass syringe distilled water empty: 6.717g
 Volume of distilled water for calibration fluid: 7.04 ml
 Concentration of calibration fluid: 309.6mg/Litre

Patient JM

Mass of plasma cuvette empty: 2.534
 Mass of plasma cuvette full with approx. 1ml of plasma: 3.508
 Initial volume of plasma: 0.974

Vol Calib	[ICG] mg/L	λ_{peak}	A 1	A 2	A 3	A 4	A 5	A 6	Mean	SD	CV (%)
10 μ L	3.146	805.6	1.22	1.08	0.961	-----	-----	-----	1.05	0.084	7.92

Offset: Zero
 Slope: 0.335 AU/mg/Litre

Patient HH

Mass of plasma cuvette empty: 2.534
 Mass of plasma cuvette full with approx. 1ml of plasma: 3.472
 Initial volume of plasma: 0.938

Vol Calib	[ICG] mg/L	λ_{peak}	A 1	A 2	A 3	A 4	A 5	A 6	Mean	SD	CV (%)
10 μ L	3.265	805.9	1.136	1.165	1.162	1.168	1.108	1.088	1.138	0.033	2.94

Offset: Zero
 Slope: 0.348 AU/mg/Litre

- **Dilute calibration fluid (5 point)**

Mass syringe ICG neat, pre: 4.297g
 Mass syringe ICG neat, post: 3.332g
 Volume ICG neat: 0.965 ml
 Mass syringe distilled water filled to approx. 30ml: 48.604g
 Mass syringe distilled water empty: 18.734g
 Volume of distilled water for calibration fluid: 29.87 ml
 Concentration of calibration fluid: 78.23mg/Litre

Patient JM

Mass of plasma cuvette empty: 2.534
 Mass of plasma cuvette full with approx. 1ml of plasma: 3.508
 Initial volume of plasma: 0.974

Vol Calib	[ICG] mg/L	λ_{peak}	A 1	A 2	A 3	A 4	A 5	A 6	Mean	SD	CV (%)
10 μ L	0.802	805.7	0.332	0.288	0.257	0.239	0.248	0.234	0.266	0.037	14.05
20 μ L	1.588	805.3	0.497	0.486	0.477	0.478	0.47	0.465	0.479	0.011	2.39
30 μ L	2.359	805.6	0.750	0.675	0.695	0.691	0.695	0.686	0.699	0.026	3.77
40 μ L	3.114	805.6	0.876	0.878	0.869	0.861	0.867	0.87	0.874	0.009	1.02
50 μ L	3.854	805.3	1.076	1.073	1.077	1.070	1.076	1.076	1.075	0.003	0.25

Offset: 0.0602 AU

Slope: 0.263 AU/mg/Litre

Patient HH

Mass of plasma cuvette empty: 2.534

Mass of plasma cuvette full with approx. 1ml of plasma: 3.508

Initial volume of plasma: 0.974

Vol Calib	[ICG] mg/L	λ_{peak}	A 1	A 2	A 3	A 4	A 5	A 6	Mean	SD	CV (%)
10 μ L	0.719	-----	0.313	0.308	0.308	-----	-----	-----	0.309	0.003	0.93
20 μ L	1.425	-----	0.513	0.512	-----	-----	-----	-----	0.513	0.001	0.14
30 μ L	2.118	-----	0.75	0.756	0.785	-----	-----	-----	0.764	0.019	2.45
40 μ L	2.799	-----	0.961	0.976	0.986	0.956	0.901	0.917	0.950	0.034	3.53
50 μ L	3.468	805.6	1.086	1.101	1.107	1.098	1.078	1.077	1.091	0.013	1.16

Offset: 0.1125 AU

Slope: 0.291 AU/mg/Litre

Observations made during the calibration experiments

Peak spectral response at approx 805 nm. Very reproducible. No change after leaving ICG standing for over 3 hours. There was no obvious effect introduced as a consequence of using heparinised sample bottles. Therefore plasma measurements are suitable for further ICG studies.

Patient JM. Difference in absorption slopes is significant. Could be due to following:

- Lack of absorbance readings using concentrated calibration fluid
- Error in zero with 2 point calibration when using concentrated calibration fluid, although the main deviation is at the high concentration end. The offset for JM is 0.0602, close to zero offset!
- 2 hours elapsed between measurements using concentrated and dilute calibration fluid. Possibly blood chemistry changed during this time.

Patient HH. Also difference in absorption slopes although the slopes are in closer agreement than with JM. Significant difference in offset: 0.1125 in dilute calibration

fluid compared with zero using 2 point calibration with concentrated calibration fluid. Errors could be due to following:

- Lack of absorbance readings using dilute calibration fluid
- 2 hours elapsed between measurements using concentrated and dilute calibration fluid. Possibly blood chemistry changed during this time.

Recommendations:

In dye reproducibility studies, calibrate via both methods in order to obtain further insight into sources of error. Concentrated and dilute calibration fluid should be prepared and used in two 1 ml plasma samples from the same patient within a few minutes. The time for which the plasma has been left standing before analysis is probably not important. It might be prudent however to check the absorption reproducibility of a sample containing ICG after 2-3 hours.

Appendix 7

Fcell derivation

$$H_{mic}/H_{sys} = H'_{mic}/H'_{sys} = \infty \text{ (constant)}$$

$$V_{rbc} = (V_b - V_{mic}) \times H_{sys} + V_{mic} \times H_{mic}$$

$$H_w = V_{rbc} / V_b$$

$$= \frac{V_b \times H_{sys} - V_{mic} \times H_{sys} + V_{mic} \times H_{mic}}{V_b}$$

$$= H_{sys} - (H_{sys} - H_{mic}) V_{mic} / V_b$$

$$= H_{sys} \times [1 - (1 - H_{mic}/H_{sys}) V_{mic} / V_b]$$

$$= H_{sys} \times [1 - \frac{(1 - \infty) V_{mic}}{V_b}]$$

$$F_{CELL} = H_w / H_{sys} = [1 - \frac{(1 - \infty) V_{mic}}{V_b}]$$

Where H= hematocrit , V = Volumes, b= whole blood , w = whole body Hematocrit,
mic = microcirculation, sys = macrocirculation , rbc = red cell mass

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POST VIVA ADDENDUM

Responses and clarifications to points discussed during the viva examination, February 2006

Introduction

- 1. The thesis would benefit from a clear enunciation of the key research questions to be addressed.**

The research hypothesis is that tools to assess volume status can be used to characterise fluid pathophysiology and determine dry weight accurately thereby preventing intradialytic haemodynamic instability during haemodialysis. The use of dynamic tests based on ultrafiltration pulses and intradialytic monitoring may allow us to understand the ultrafiltration pathophysiology. Insight into these mechanisms may then provide the opportunity of individualising the ultrafiltration prescription.

- 2. Expansion of the sections relating to patients selection. This is crucial in terms of overall applicability of observations in this fairly highly selected patients group**
 - a. Why no HDF patients used, does this select out a certain phenotype?**
 - b. Use of high flux HD, ? effects on adipokine removal etc**
 - c. RRF in this patient group**

Diffusive and convective dialysis occur simultaneously in high flux dialysis and the components cannot be separated during the treatment or its effect distinguished on haemodynamic parameters. Predominant haemodiafiltration however may have other effects due removal of middle molecules e.g ADMA or differential sodium removal or temperature effects. The initial studies as part of this research was performed excluding HDF patients to avoid other confounders that may limit comparison with more standard and more widely used high flux dialysis. Most blood volume experiments in this research studies patients as their own controls. The effect of RRF would have an overall haemodynamic consequence and has been investigated in individual studies where relevant. Diffusive effects on relative blood volume may be a result of altered vascular resistance, changes in regional blood flow distribution and osmolality changes, The latter has a minimal effect on blood volume monitoring using ultrasonic method as explained in the Methods section, The other influences were minimised by achieving a stable blood volume profile prior to each ultrafiltration pulse applied in an intermittent fashion.

- 3. An expansion of the discussion of the role long term salt and water overload might play in the pathogenesis of increased CV events.**

Today, increased aortic stiffness has been recognized as an important determinant of increased cardiovascular risk. Sodium/volume overload is related to aortic stiffness. Angiotensin-converting enzyme inhibitors administered to dialysis patients failed to normalize, aortic pulse wave velocity as an index of vascular stiffness. Complete normalization was only achieved with combined volume control plus

pharmacological blockade of the Renin Angiotensin System. This is one of the few documented examples showing the importance of salt/volume in target organ malfunction. One of the mediators is oxidative stress. Oxidative stress is also one of the hallmarks of renal failure. Numerous experimental studies documented induction or amplification of oxidative stress by salt, and reversal of organ damage in salt-sensitive models of tissue injury by antioxidative intervention (1). It has been amply documented that left ventricular hypertrophy and other characteristics of the vasculature such as pulse pressure and vascular stiffness are influenced by salt intake independently of blood pressure and may amplify the response to high blood pressure (2). A study in Finland showed that high salt intake translates into higher cardiovascular mortality in the general population (3). The effects of angiotensin II and aldosterone are known to be amplified by a high salt intake. Some direct and blood pressure-independent effects of high salt intake have been identified within 1 day and without change in blood pressure, eg high salt intake causes increased transforming growth factor expression in kidney and aortic endothelial cells. The blood pressure response to high salt has been shown to be determined by the availability of nitric oxide, and interestingly, endogenous nitric oxide synthase is stimulated by transforming growth factor. Salt, perhaps as a result of increased shear stress, has numerous effects on endothelial cell mitogen-activated protein kinase and SMAD signaling making endothelial cells hot candidates for salt-induced organ damage (4).

4. An expansion concerning the relative merits of the differing definitions of HT in HD patients.

There are 3 ways in which we can assess the level of BP in a hemodialysis patient. Blood pressure can be obtained during, before, and after dialysis by the dialysis staff, at home by the patient, or by an automatic ambulatory BP monitor. Guidelines suggest that predialysis and postdialysis BPs should be <140/90 and <130/80 mmHg, respectively. These results fail to provide solid data to back the K/DOQI guideline recommendations regarding BP goals in hemodialysis patients. Better methods are needed for the assessment of BP in hemodialysis patients for clinical decision making. The hemodialysis population is associated with a very low survival rate, with cardiovascular disease accounting for most of the increased mortality. Recent observational studies demonstrate a paradoxical relationship between lower blood pressure and increased mortality (5). Hypertension treated with antihypertensive medications unequivocally reduces cerebrovascular risk, but demonstration of a survival benefit for cardiovascular mortality has proven more difficult to demonstrate. Increased pulse pressure increases arterial wall stiffness and afterload, and decreases coronary perfusion. The disproportionate representation of diastolic dysfunction and coronary artery atherosclerosis may explain why increased pulse pressure is associated with higher cardiovascular risk for the dialysis population. Optimum blood pressure control has not been established, due to a lack of prospective studies targeting blood pressure reduction.

5. A further discussion of other factors in HT other than volume expansion (eg adipokines, Na loading of resistance vessels, sympathetic overactivity). The complex role HT and lack of direct correlation with mortality needs some further discussion.

High BP is a major risk factor for atherosclerotic cardiovascular disease (ASCVD) mortality in the general population. The high prevalence of hypertension among hemodialysis (HD) patients may contribute to the observed excess of ASCVD morbidity and mortality. Thus, it is important to identify the ideal BP target in this high-risk group. The National Kidney Foundation's (NKF's) Kidney Disease Outcomes Quality Initiative (KDOQI) and JNC VII have recommended a target BP <130/80 mmHg for patients with chronic kidney disease stages I to IV. NKF KDOQI has recently recommended pre- (<140/90 mmHg) and post-HD (<130/80 mmHg) BP targets. The evidence supporting this recommendation was graded as weak because it was extrapolated from the general population. The only prospective study in a dialysis population demonstrated that a BP \leq 140/90 was associated with a decreased risk for left ventricular hypertrophy but an increased mortality risk .

A "U"-shaped relationship exists between postdialysis SBP and mortality in a prevalent cohort of HD patients. Mild to moderate elevations in predialysis SBP were not associated with significant increases in ASCVD and all-cause mortality. These observations were subsequently confirmed by other investigators. High postdialysis SBP and low pre- and postdialysis diastolic BP (DBP) were associated with increased mortality. (6) Port *et al.* reported that predialysis SBP <110 mmHg was associated with increased mortality. (6) In contrast, other investigators did not observe an association between BP and mortality. To summarise, the studies that have been conducted among hemodialysis (HD) patients have yielded conflicting data on the relationship between BP and mortality. (6)

6. More discussion concerning the published data on the heterogeneity in haemodynamic response to RBV within and between patients.

There is conflicting evidence on the potential benefits of relative blood volume (RBV) monitoring.(7,8) The studies have so far looked at crude estimates of relative blood volume change (percentage change) but failed to define other important characteristics in the RBV response of individual patients to fluid removal, their relationship with other pathophysiological variables such as compartmental changes, and their relationship to absolute measures. Moreover RBV may well be affected by cardiovascular compensation and pathophysiological changes that have not been scrutinised. The thesis aims to explore these parameters and compare relative and absolute measures to obtain a better understanding of changes that occur during ultrafiltration.

7. Expansion of the section pertaining to Na removal in HD (the pitfalls of measurement etc.

Sodium crosses dialysis membranes by means of two mechanisms: convection and diffusion. Thus sodium removal can be increased both by applying higher ultrafiltration volumes and by lowering dialysate sodium concentration. While ultrafiltered fluid is slightly hypotonic due to the Gibbs-Donnan equilibrium, large ultrafiltration volumes do not substantially change the so-called “hydrosodium balance” (i.e., the relation between sodium and water in the body). The principle of diffusive sodium removal is, however, more complicated. In both blood and dialysate, a significant, but differing, percentage of sodium is bound to anions and unavailable for diffusion. In plasma water, it is assumed that ± 7 mmol/L of the total sodium concentration of ± 150 mmol/L sodium is in a complexed form versus ± 4 mmol/L in dialysate. It has been shown that no net diffusive sodium transport between blood and dialysate occurs when sodium in the plasma, as measured by flamephotometry, is ± 2 mmol/L lower than dialysate sodium concentration (9). This roughly corresponds to a difference in sodium activity between plasma water and dialysate of 4 mEq/L and to a Donnan effect in HD of 0.97. However, due to protein coating of the dialysis membrane, the Donnan effect may be unpredictable and can be larger than theoretically assumed. Diffusive influx may occur with supraphysiologic dialysate sodium concentrations and in patients with low predialytic plasma sodium concentrations.

8. A further discussion on ideal target weight, how we can define it, and that the pragmatic definition as it being the weight that IDH does not occur may not give an optimal weight wt expansion of TBW.

Dry weight, post dialysis weight, target weight, ideal weight, right weight are terms coined to define a state of euvolemia in a dialysis patient. This is prescribed in clinical practice by absence of signs of hyper and hypovolemia – the latter being a more sensitive tool as it is easy to measure objectively (intradialytic hypotension). There are several caveats to this assumption as intradialytic hypotension may be governed by prescription variables i.e ultrafiltration rate or intrinsic factors independent of the fluid status and specific to each patient. Moreover dry weight is a constantly changing parameter and linked to lean body weight changes which can be unrelated to fluid status. The relationship is complex and poorly understood. factors independent of the fluid status. More importantly in silent hypervolemia, patients may remain asymptomatic and yet above their dry weight. This thesis attempts to address some of these variables and their interaction during ultrafiltration.

Methods

1. **More detailed description of method for Kt/V measurement.**
2. **?Any method for recirculation studies, and were QaS MEASURED.**
3. **More needs to be said about the potential limitations of BIA (eq frequency, segments studied, reliability, use of regression equations from normal populations etc etc)**
4. **More detail in method of exactly how BIA was performed (posture etc)**

KT/V was assessed using Tattersall formula using predialysis sample, postdialysis sample with rebound quantification and a predialysis sample taken on the next dialysis session.

The formula used for 2 pool Kt/V was:

$$Kt/V \text{ (2-pool)} = Kt/V \text{ (single-pool)} * (t/(t + tp)) \quad (10)$$

BTM (Fresenius) recirculation measurements were part of routine clinical practice. Whole body multifrequency BIA used in this study measures fluid volumes by comparing resistivities and extrapolating volumes from algorithms and software that have been validated in the general population. Although resistance can be converted to absolute volumes , this relies on derived equations in specific populations or the application of complex physiologic principles of emulsion theory that may introduce unknown errors. Studies in the dialysis patients have however shown excellent reproducibility. Measurements were made on the nonfistula arm in case of dialysis patients in supine position.

Chapter1

1. **Patient choice, ? limitations of only using MWF day shift type patients.**
2. **More detail on the discussion of the importance of dipping in the ABP data.**
3. **Need to mention dialysate conductivity and whether any Na profiling was used.**

The patients selected for the study in Chapter 1 were haemodialysis patients in both MWF and TTS shifts. Early morning and late evening shifts were excluded for logistical reasons. The dialysate conductivity was fixed at 140 Ms/cm with no profiling. The lack of nocturnal dipping was particularly noticeable in a large group of patients. Persistent abnormal BP variability is known to be a strong correlate of a dilated heart on dialysis, independent of the BP level.

Chapter 2

1. **What were the dietary recommendations?**
2. **Discussion concerning the lack of any other body composition analysis other than BIA.**
3. **Did BIA measured TBW changes refelect observed changes in weight?**

4. **The PD data does not sit comfortably here. There is inadequate data on the PD regimes etc to allow much in the way of comparative insights ? remove.**
5. **The lack of normal / suitable CKD controls make discussions of malnutrition being late less valid.**

Subjects were randomly selected from an outpatient's clinic for a cross-sectional prospective nutritional assessment to compare direct and modeled estimates of nutritional status in patients with renal insufficiency. All patients received standard dietary advice, which for patients with Stage 5 CKD on dialysis consisted of salt restriction to 4-6 g/d, no protein restriction, potassium and phosphate restricted diet and a set target weight assessed routinely

Techniques for body composition methods that can be performed repeatedly especially during dialysis are lacking. Bioimpedance provides a reasonable estimate of the fluid compartments with excellent reproducibility. Moreover their ease and applicability make it the only suitable noninvasive device for objective measurements of fluid compartments during a clinical dialysis session. Body weight and ultrafiltration volume changes closely tracked changes in ECW (Fig 4, 5) and TBW. As multifrequency measurements were made, ECW was felt to be a better marker of the fluid compartments studied.

The observational data on PD and HD was primarily used to assess discrepancies in fluid compartments and nutritional parameters between the twodialysis modalities and with the CKD population. The PD population studied was almost identical in numbers with the HD cohort and revealed interesting findings. However such a comparison of modalities is perhaps too simplistic and subject to several caveats.

Chapter 4

1. Were there any data on sx without IDH?

The study endpoint Hypotension was defined as a systolic BP < 90 mm Hg or a fall in mean BP of > 30 mmHg associated with symptoms of suggestive of hypovolemia, such as dizziness, faintness, nausea or cramps. Hypotensive pulses analysed were those which were acceptable as per the definition. Any other symptomatology was not included in the analysis.

Chapter 5

1. Are there any data on the reproducibility of RBV decay within individuals.

In Chapter 5 studies were performed only once for each individual. However in the preceding chapter 4 it was observed that the rate of change in blood volume in the first and second UF pulses was significantly correlated with the volume of the ultrafiltrate (boli1 $r = 0.90$, $p < 0.01$; boli2 $r = 0.89$, $p < 0.01$). This correlation was also observed in the later uf pulses (prefinal pulse $r = 0.80$, $p < 0.01$; final pulse $r = 0.42$ $p < 0.05$) although at lower significance. (Fig 5) By stepwise multiple regression analysis ($R^2 = 0.743$) the determinants of ΔRBV_{UF} were UFVs ($p < 0.001$), patient specific factors ($p = 0.015$), and distance from dry weight ($p < 0.041$). It appeared that

the UF volume and hydration status were the two dominant effects and could be reproduced within the same patient, with high reproducibility of the decay constants. The refill patterns, in the absence of UF, were also characteristic and determined by individual patients but had wide interindividual variability and poor reproducibility.

Chapter 6

- 1. Very small study.**
- 2. Need more justification of comparison with 37.4 rather than standard temperature practise.**
- 3. Was there any data on other sx (partic temp tolerability)?**
- 4. Did T effect RBV response**

The chapter described a pilot study on a small number of subjects who acted as their own controls to examine the effect on blood volume parameters of a prescription variable i.e. dialysate temperature. The prescribed temperature were 35 C for cool dialysis and 37 C for warm dialysis sessions for each subject and set at the start of each dialysis session. The venous temperature data was recorded every min and downloaded throughout the dialysis treatment on a real time database. The average venous temperature recorded by the BTM for all warm dialysis sessions averaged at 37.4 ± 0.2 C and the average temperature recorded for all cool sessions were 35.3 ± 0.3 C. ($p < 0.000$) Table 4. The slight discrepancy between the prescribed dialysate temperature and the averaged venous BTM readings are possible explained by 1) methodological differences 2) heat exchange in the extracorporeal circuit 3) inherent variability in the thermostat control settings within the machine. The primary end point of a differential heat exchange was achieved to analyse the result. The other alternative would have been to prescribe the net thermal flux rather than a fixed dialysate temperature. At the time of setting the study design I felt that the latter approach would be a more artificial setting and therefore more difficult to apply in clinical practice. Patients were not blinded to the warm or cold sessions, hence symptoms more difficult to interpret. However no study had to be terminated due to intolerability.

It is hypothesized that those with lower predialytic temperatures would be prone to higher net heat gain and therefore predisposed to greater blood volume instability. The effect of predialytic temperature on RBV responses in different subjects were not investigated as there was insufficient study subjects to do the analysis.

Conclusions

Needs a more definitive statement of the insights that this series of investigations achieved, o balance the greater clarity of how the research questions are framed at the start of the thesis.

The aim of the research was to characterise the pathophysiology of ultrafiltration (UF) during haemodialysis (HD) using a range of assessment tools. The hypothesis was that objective monitoring would facilitate accurate estimation of dry weight (DW) and help optimise UF. The initial studies to characterize fluid compartments and their

relationship with traditional clinical measures of hydration status (BP, body weight and composition) revealed a complex relationship with several confounding factors which are specific to the dialytic cycle, uremic environment, nutritional status and is patient specific, highlighting the need for objective characterisation of the fluid compartments and its alterations during fluid removal. Multifrequency bioimpedance confirmed the value of tracking fluid shifts repeatedly and reliably to quantify the extracellular compartments. We then examined changes in the blood volume compartment induced by UF with approaching dry weight. It was evident that relative blood volume changes induce characteristic changes in the RBV profiles that can predict DW and haemodynamic stability but require sophisticated analysis and algorithms. The most useful of these parameters was a quantity we have designated the “refill divergence”, which is a measure of the deviation from linearity of the RBV curve during a step UF. Refill divergence and τ_{UF} , measures of the deviation from linearity of the RBV curve during UF, predict hypotension and thus more helpful in predicting the onset of haemodynamic instability as dry weight was approached. Monitoring deviation from linearity may allow improved haemodynamic stability and attainment of optimal post-dialysis weight. These blood volume parameters could be used to study the influence of several variables that have haemodynamic influence. Blood volume changes in the absence of UF (refilling) during dialysis remained widely variable. Approaching dry weight the RBV decline during UF switched from exponential to linear decay, two pool to single pool behaviour, probably indicating failing vascular refill. The latter can only be quantified using absolute blood volume measurements.

I then undertook a series of experiments to analyse and explore blood volume responses during UF using directly measured absolute blood volumes. I pioneered a technique of repeated absolute blood volume estimates during dialysis using Indocyanine green. The purpose of this analysis was to examine directly measured plasma volume, blood volume changes and refill responses within compartments of the extracellular volume and their relationships with vascular stability during profiled intermittent ultrafiltration during haemodialysis. The use of tracer (Indocyanine green) and density (Relative blood volume) method synchronously allowed us to characterise how the circulation regulates and redistributes blood volume in response to ultrafiltration. The method enabled quantification of absolute refill rates and factors causing its variability, the most significant being microcirculatory adjustments induced by ultrafiltration. This research established that the tools for monitoring blood volume and fluid compartments can be used to objectively define approaching dry weight and avoid impending haemodynamic instability. The research indicated that an understanding of the microcirculatory changes may provide further insight into the mechanism of intradialytic hypotension and its prevention in haemodialysis.

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